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JOURNAL OF ANATOMY

STUDIES ON THE AREA VASCULOSA OF THE EMBRYO CHICK

II. THE INFLUENCE OF THE CIRCULATION ON THE DIAMETER OF THE VESSELS

By A. F. W. HUGHES
Sir Halley Stewart Research Fellow

From the Strangeways Research Laboratory, Cambridge

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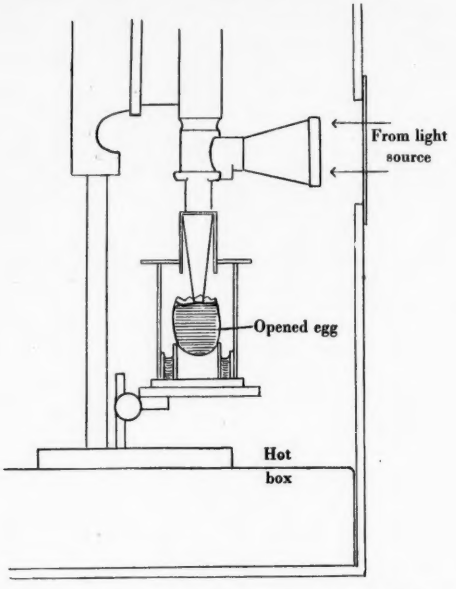
INTRODUCTION

THE first paper of this series (Hughes, 1935) was an attempt to continue Thoma's work of the effects of the blood stream on the vessels of the chick area vasculosa. In general, arteries and veins are formed from smaller vessels, usually in a capillary network. The circulation of blood is believed to be responsible for the enlargement of the capillary vessels, and Thoma suggested a relationship between the flow of blood in a vessel and the diameter of the vessel, which is as follows: The increase in size of the lumen of a vessel, which is proportional to the growth in surface of the vessel wall, is dependent upon the rate of the blood flow.

The evidence which he offered for this assertion is concerned with the manner in which the vitelline artery is first formed in the chick blastoderm (Thoma, 1893). This evidence is briefly as follows: After the heart has begun to beat effectively, all the blood flowing from the aortae of the embryo to the area vasculosa runs through a narrow band of the vascular network on each

side, in which the rate of flow is therefore much more rapid than elsewhere. These regions are soon transferred into the vitelline arteries by the enlargement and fusion of the meshes of the network.

The first paper of the present series (Hughes, 1935) is chiefly concerned with the detailed development of the vitelline artery, but neither in this paper, nor in those of Thoma are there any measurements of the comparative rates of flow in different regions of the area vasculosa, and thus no direct proof of Thoma's principle is attempted. Recently, the rate of flow of the blood in different regions of the area vasculosa has been measured, and quantitative data concerning the validity of Thoma's hypothesis have been obtained, which it is the purpose of this present paper to describe.



Text-fig. 1. Diagram of apparatus, showing egg chamber mounted on indirectly illuminating microscope, which is enclosed by the hot box.

METHODS

(1) *Observation*

There are two methods for observing living chick blastoderms, one is to remove the blastoderm from the yolk and grow it *in vitro* in a suitable culture medium. In the other method, the blastoderm is left inside the yolk; part of the shell is removed, and the egg is incubated in a moist atmosphere (Romanoff, 1931). The second of these methods has been chosen for the present work, since it was necessary to observe a single blastoderm over long periods. Eggs opened during the second day of incubation and placed in a suitable moist

chamber, frequently continue to develop for a further 7 days, and occasionally longer. By this means, therefore, it is possible to study a wide range of blood vessels, in which growth and differentiation are taking place extremely rapidly, and the method thus offers considerable possibilities in the study of vascular development.

(2) *Estimation of the velocity of the blood stream*

The rate of blood flow to which Thoma's hypothesis refers is expressed in volumes of blood flowing in unit time. Cannulation methods which are inapplicable to embryonic vessels are necessary in order to measure directly the rate of blood flow. In this work, the velocity of the corpuscles seen in motion under the microscope has been measured. To calculate the rate of flow of the blood from the velocity of the corpuscles, two further sets of data are required: one, the general relationship between the position of the corpuscles along the radius of the vessel and their velocity, and secondly, the actual position on the radius of the vessel of the corpuscles whose velocity we are able to measure. For a homogeneous fluid flowing in a tube under certain circumstances, the first of these relationships can be derived from Poiseuille's law connecting the rate of flow and the viscosity of a fluid flowing in a tube with the difference of pressure at the two ends. It would not be justifiable to apply the Poiseuille formula to the vessels of the area vasculosa without previous demonstration of its validity in these circumstances.

Viewing the vessels by indirect illumination, the corpuscles which we see in motion are the outermost ones in the vessel. In the larger vessels, these corpuscles appear very close to the vessel wall, and no layer of corpuscle-free plasma lining the vessel wall can be seen; under the magnifications which have been used in this work it is not possible to measure the actual distance between these corpuscles and the vessel wall, and small differences in this quantity would make very large differences to the rate of flow calculated on Poiseuille's formula.

For these reasons, we have used the velocity of the superficial corpuscles itself as our index of rate of flow and have called it "marginal velocity". The exact manner in which blood flows through the vessels of the area vasculosa is now being investigated by means of slow-motion cinema pictures, but the present paper describes only the correlations which have been observed between rate of change in diameter of the vessels, and the marginal velocity.

The marginal velocity is measured by means of an apparatus in which an external point of light reflected into the field of the microscope by means of a camera lucida is made to traverse the field at such a speed that the point appears to travel at the same rate as the corpuscles in the blood vessel. The point source of light is attached to a pencil which is moved across a band of paper, which itself moves at right angles to the direction of motion of the pencil. A series of oblique lines on the paper are thus drawn, and from the slope of these

lines, the velocity of the paper band, and the magnification of the microscope, the velocity of the corpuscles can be calculated.

About ten tracings of corpuscle movement were made at a time for each vessel, and the resulting velocity values were averaged. Table I gives the results of a series of measurements of corpuscle velocity in a single vessel made at intervals of a few minutes, with two different magnifications. Under the higher of these magnifications, the blood stream appears so rapid that it is difficult to follow it accurately with the pencil point. Accordingly, the values for corpuscle velocity tend to be lower and more divergent with the higher magnification. The lower magnification has therefore been used generally for measuring corpuscle velocity in this work. The degree to which such results are comparable may be judged from Table I, in which the lowest and highest average values for corpuscle velocity obtained with the lower magnification differ by 32 per cent.

Table I. *Measurements of marginal velocity on one vessel*

Time	Heart rate	Objective	Average marginal velocity	Range	Percentage of mean
8.55	136	3·8	416	390–460	94–110
9.12	136	3·8	423	360–500	88–118
9.30	150	3·8	416	350–490	84–118
9.45	144	3·8	476	445–540	93–114
10.5	—	11·0	270	220–290	82–108
10.25	—	11·0	446	374–540	84–120
10.30	136	11·0	360	290–420	81–116
10.40	—	3·8	503	480–540	95–108
10.55	150	3·8	551	400–630	73–114

Average of nine series: 423

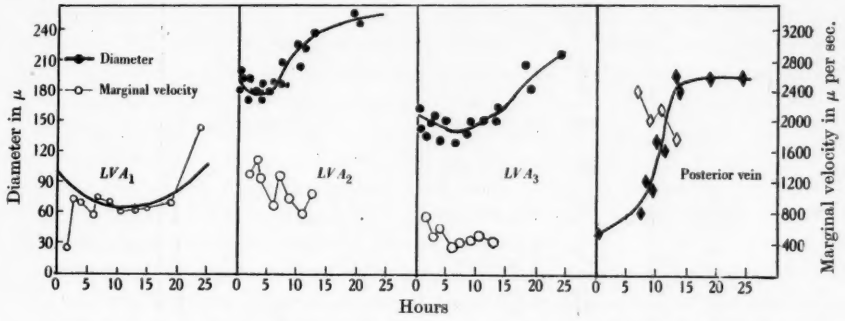
MEASUREMENT OF DIAMETER AND MARGINAL VELOCITY

(1) *Arterial branches*

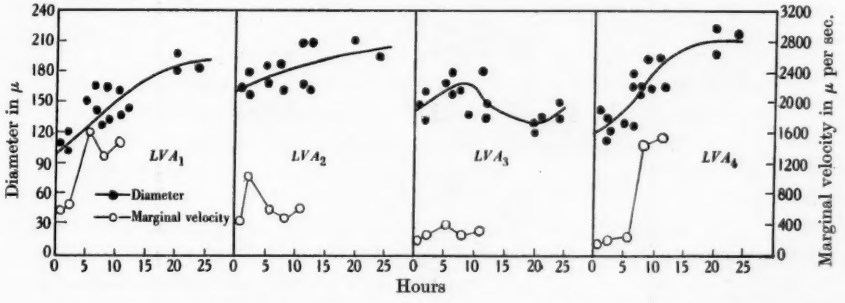
The marginal velocity and the diameter of each of the main branches of the vitelline artery of one side of the area vasculosa were measured at intervals; observations were begun early in the second day of incubation, and the differences between the various branches were used to study the relationship between marginal velocity and rate of change in diameter.

The changes in relative importance of the branches of a vitelline artery follow certain lines which are determined by the general changes which take place within the area vasculosa. During the first 10 hours of circulation, blood is returned to the embryo mainly through the only veins which have so far developed, namely the anterior pair which are part of the marginal sinus. Consequently, the greater part of the blood issuing from the vitelline arteries flows anteriorly, in the anterior branches of the main artery. The main flow from the vitelline artery does not run directly anterior, however, for the vessels along the direct path nearest the embryonic axis remain in a contracted state for the first few hours after the circulation has begun (Hughes, 1935) and must

therefore offer a greater resistance to the blood flow than a less direct path through wider vessels. In AV_1[1] (Plate I, fig. 2), the second of the three branches of the left vitelline artery (LVA_2) carries most of the flow throughout the whole period of observation; in AV_2 (Text-fig. 3), where the left vitelline artery has four branches, branch (LVA_2) is the largest in diameter at the time when observation begins. Up to this period, therefore, those vessels in which the flow has been greatest have acquired the larger diameter.



Text-fig. 2. Relations between marginal velocity and diameter of the branches of the left vitelline artery and of the posterior vein of AV_1. The points for the diameter of LVA_1 are omitted for the sake of clearness.



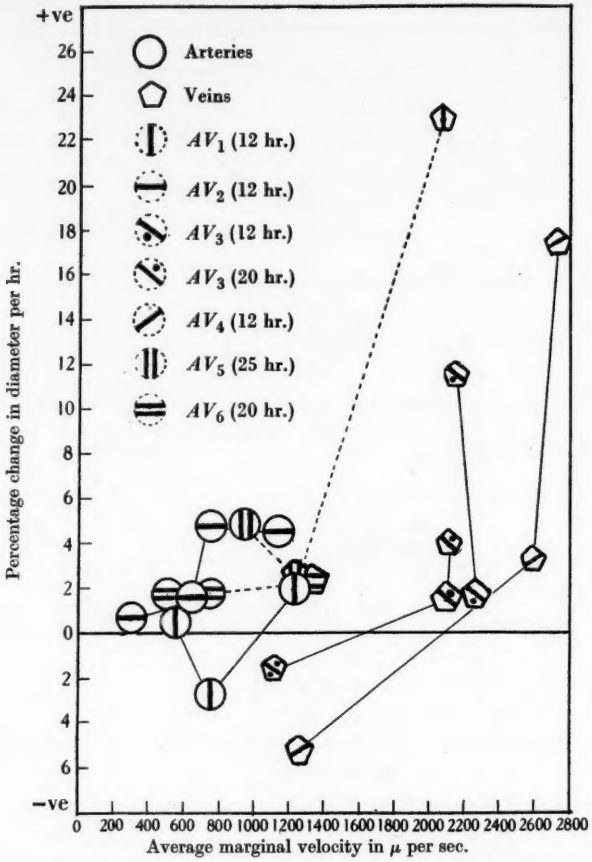
Text-fig. 3. Relations between marginal velocity and diameter of the branches of the left vitelline artery of AV_2. The same symbols are used as in text-fig. 2.

The development of other return paths soon modifies these relationships. A direct path is formed from the posterior region of the area vasculosa along the left side of the embryonic axis, and over the left vitelline artery. This is the posterior vein. This path is formed by capillaries already present on either side of the artery, but it is not completed until these are connected dorsal to

[1] The following abbreviations are used in this paper. The series of individual area vasculosae examined in the living state are referred to as AV_1, AV_2, etc. In each specimen the branches of the left vitelline artery are numbered antero-posteriorly, as LVA_1, LVA_2, etc.

the artery by newly formed vessels, which originate in the manner described in the previous paper of this series (Hughes, 1935).

The establishment of a direct return path from the posterior region of the area vasculosa tends to increase the arterial flow to that region by diminishing the peripheral resistance. Accordingly, a smaller share of the total arterial



Text-fig. 4. Percentage change in diameter plotted against average marginal velocity for veins and arteries in six individuals, over the periods indicated. Points referring to arteries and veins of the same individual joined by dotted lines; points referring to similar vessels of the same individual joined by continuous lines.

flow on each side is distributed through the anterior branches. In AV_2 the posterior venous path had formed after 5 hours of observation, and at this time the marginal velocity in the posterior branch, LVA_4 increased sharply, as shown in Text-fig. 4.

By the time the posterior venous flow has begun, a further series of veins are differentiating, which, like the posterior vein, are formed through the isolation of the arteries from the surrounding capillaries, which then join together across the dorsal surface of the arteries. A lateral system of veins develops over the branches of the vitelline arteries, which ultimately completely cover the main branches. In low-power photographs, it is possible to see the lateral veins only where they do not follow the same course as the pre-existing arteries. Thus in figs. 7 and 8 of Plate I, the vessels (*vv*) which develop rapidly during the latter part of the period of observation are part of the lateral vein system. The venous network which runs alongside each artery provides a more direct return path for the capillary area supplied by that artery than the main anterior and posterior veins. A rapid flow in this venous network begins in the reverse direction to that in the underlying arteries, and the capillaries of the network rapidly enlarge to form a system of venae comitantes which largely follow the same path as the arteries. The greater the flow carried by an artery at the time of the formation of this venous network, the more rapidly will an accompanying vein differentiate alongside the artery.

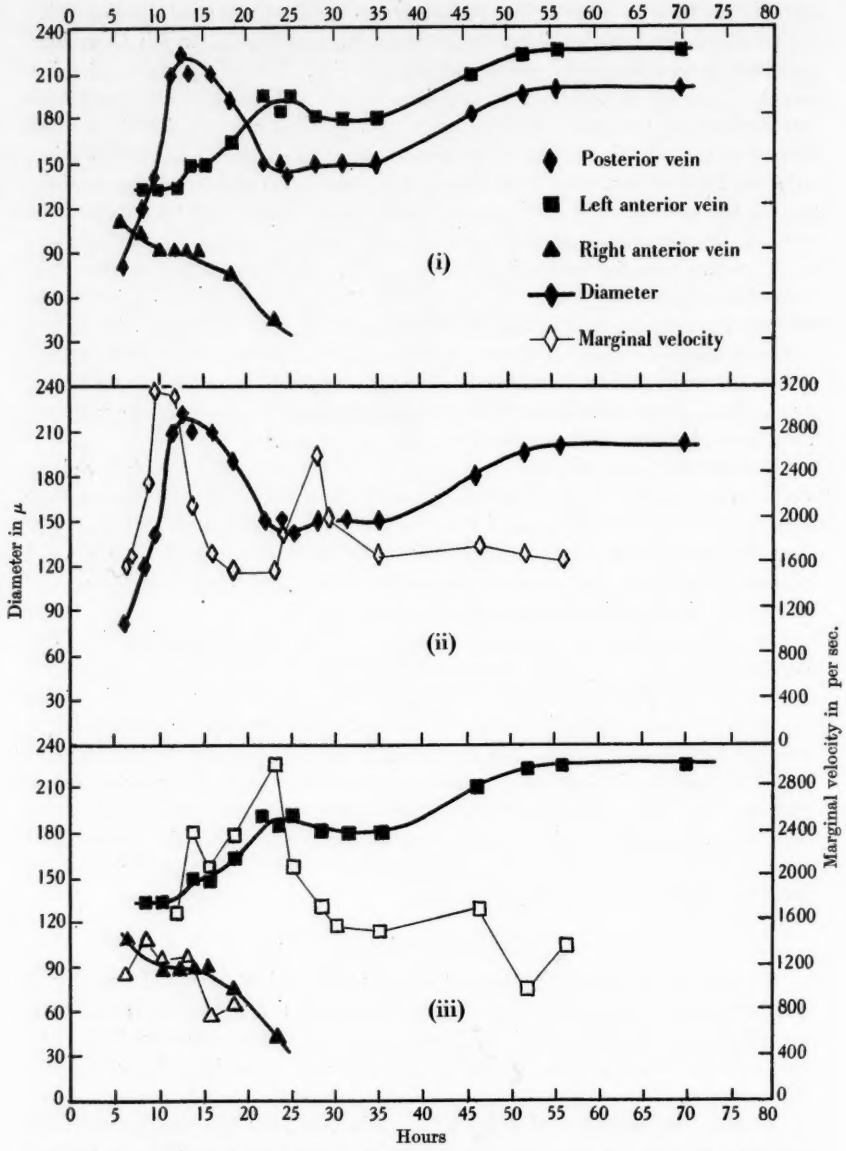
In both AV_1 and AV_2 the main branches of the arteries were obscured by veins 20 hours after observation had begun, with the exception of the smallest vessels, which were LVA_1 and LVA_3 respectively. Consequently, it was only possible to measure the marginal velocity in all the branches over a total period of 12 hours, and hence comparison of marginal velocity with rate of change in diameter is only possible over this period.

In order to compare the ranges of values in these two quantities with both the biological variation and the size of the errors involved in these measurements, the correlation diagram shown in Text-fig. 5 was constructed in which percentage change in diameter over the period of observation is plotted against average marginal velocity. For the arteries alone, the points show a general trend of increase in rate of enlargement with increase in average marginal velocity, but this correlation becomes far clearer when the venous points are considered in addition, since veins show a much wider range in these values than do the arteries. For this reason, veins received the greater share of attention during the course of this work.

(2) *Veins*

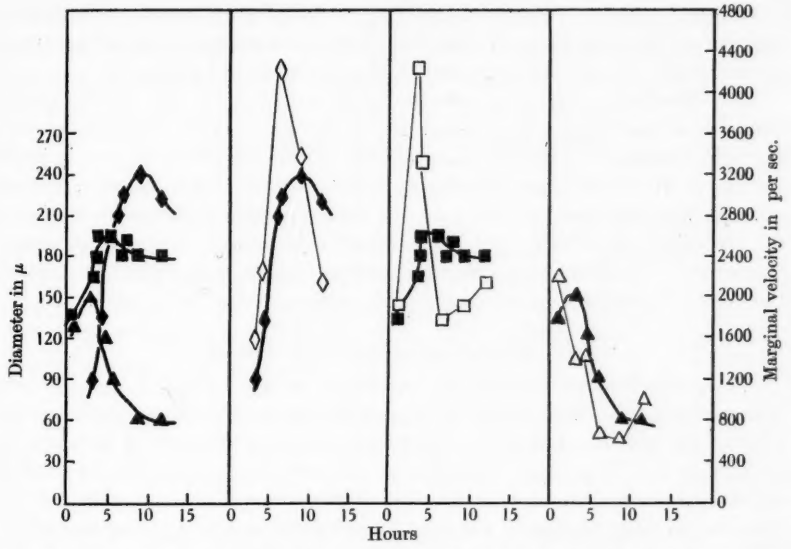
In order to compare the rate of enlargement and of marginal velocity in different veins in the same blastoderms, three veins were chosen, namely the posterior vein of which the development has already been described, and the two anterior veins, whose subsequent development is particularly suitable for this purpose.

During the second and third days of incubation, the gap between the anterior veins of the area vasculosa gradually narrows, and is finally bridged by means of anastomoses between these vessels (Plate III). At this stage the anterior region of the area vasculosa is a continuous network, and the vessels



Text-fig. 5. Relations between marginal velocity and diameter of main veins AV_3: (i) diameter only for the three veins; (ii) diameter and marginal velocity for the posterior vein; (iii) diameter and marginal velocity for the two anterior veins.

which carry most blood back to the heart from the anterior region are those which offer least resistance, irrespective of whether these are the original right or left anterior veins. Usually one path offers less resistance than all others, and in carrying a greater flow of blood than other paths, gradually enlarges to form the final anterior vein, which in common with other vessels which differentiate from a vascular network does not originate in an identical manner in different individuals. Popoff (1894) states that the left anterior vein becomes the final anterior vein, but Grodzinski (1935), who has followed these changes in the living area vasculosa, found that out of ten blastoderms, the final anterior vein was formed from the left vein in four, from the right in three,



Text-fig. 6. Relations between marginal velocity and diameter of main veins of AV_4: (i) diameter only, for the three veins; (ii), (iii) and (iv) diameter and marginal velocity for posterior, left anterior, and right anterior veins respectively. The same symbols are used as in Fig. 5.

and from parts of both in the other three. In the photographs of AV_8 (Plate III), the proximal part of the original left anterior vein becomes the proximal part of the final anterior vein, but the distal part of the latter is formed from an anastomosing vessel, between the original anterior veins, both of which ultimately become part of the general area vasculosa in this region. In these veins therefore, there is considerable scope for the study of rate of change in diameter.

Two sets of results are given here of measurements of all three veins in the same area vasculosa. In AV_3 (Text-fig. 5), both the diameter and marginal velocity of the posterior vein increased rapidly for the first 12 hours of observation. Then the marginal velocity suddenly decreased, and the diameter of the

vessel began slowly to diminish, until at 25 hours, the diameter again began to increase following a temporary increase in marginal velocity. The course of events was similar for the left anterior vein, which became the final anterior vein, but here the initial increase in diameter was less sudden, and was followed by a less strongly marked period of decrease. In the right anterior vein, the marginal velocity was less than in the other veins when observations began, and the diameter of the vein slowly decreased, as the marginal velocity tended to drop still further.

In AV_4, where observations extended only over 12 hours, the earlier of these events are seen extremely clearly. Both marginal velocity and diameter at first increased rapidly for both posterior and left anterior veins and the drop in marginal velocity clearly preceded the change in direction of the diameter-time curve. The right anterior vein did not begin to decrease in diameter until the marginal velocity had been diminishing for at least 4 hours.

The exact relationship between marginal velocity and rate of change in diameter is clearly not a simple one. In the first phase of increasing diameter, the vein continues to enlarge only as long as the marginal velocity is itself increasing. When the marginal velocity no longer continues to increase, instead of increasing at a much slower rate, the vein decreases in diameter. Thus for the posterior vein of AV_3, the marginal velocity and diameter are the same respectively 16 and 40 hours after observations begin, yet at the former time the vein is decreasing in diameter, and increasing at the latter time.

(3) *Comparison of arteries and veins*

It remains now to consider the correlation between marginal velocity and rate of change in diameter for both arteries and veins. The values for the percentage increase in diameter of veins over a whole period will largely depend upon how great a proportion of this period is occupied by the initial rapid increase in diameter; during this initial stage, the percentage rate of increase per hour may be as much as 40; and this value will diminish progressively as more of the subsequent stages fall within the period considered. On the other hand, the arteries change in diameter more gradually, and no constant phases in their development over this period can be distinguished. In Text-fig. 4 most of the points relate to a period of about 12 hours, although some are calculated for a 20-hour period. For AV_3, points are given for the three veins for both periods.

Two main conclusions can be drawn from Text-fig. 4. First, that the numerical relationship between average marginal velocity and percentage change in diameter may be quite different for corresponding vessels in different blastoderms. If we compare the anterior veins of AV_5 and AV_6 with those of AV_3 and AV_4, we see that they all increase approximately equally in diameter over the whole period, but the average marginal velocity in the former group is about 1300 μ per sec., and in the latter about 2400 μ per sec. The same average marginal velocity resulted in rapid decrease in diameter of the right

anterior veins in the latter group, and increase in diameter of the left anterior veins in the former group.

These biological variations, however, do not prevent a further conclusion being drawn, namely that in comparing veins and arteries generally, for a given percentage change in diameter, the average marginal velocity is higher for veins than for arteries. An average marginal velocity of between 400 and 1000 μ per sec. in the arteries results in no change in diameter; the corresponding value for veins is between 1600 and 2200 μ per sec. In AV_5 and AV_6, observations were made in each blastoderm for both veins and arteries which increased in diameter at similar rates. In AV_6, these rates were almost the same for both veins and arteries, and the average marginal velocity for the former was about double that of the latter; in AV_5, the rate of increase in diameter of the artery was higher than that of the vein and the average marginal velocity was lower.

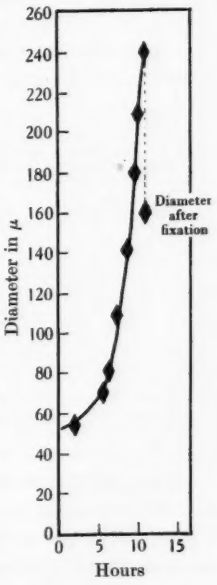
THE GROWTH OF ENDOTHELIUM

(1) *In rease in vessel diameter*

During the first phase of the development of a vein from a capillary, the rate of increase in diameter may be as much as 40 per cent of the original diameter per hour. This rate is far greater than the rates at which embryonic organs generally increase in size. The area of the endothelium of a vessel increases at the same rate as the diameter. It is first necessary to know whether this rate is also the rate of increase in number of the endothelial cells, or whether the increase in area of the vessel wall is due to any extent to increase in the size of the endothelial cells.

To investigate this point, the diameter of a living vessel was measured over a certain period, which ended at fixation. The size of the cells in the vessel wall was estimated subsequently from serial sections, and expressed as the average area of endothelium to each endothelial nucleus.

The first area vasculosa which was studied in this way was AV_7, in which the increase in diameter of the posterior vein is shown in Text-fig. 7 and in Plate II. In the fixed state the diameter of the vein is 160 μ and the value for the area of endothelium per nucleus is 303 sq. μ. For a capillary on the opposite of the embryo, 40 μ in diameter and similar to that from which the posterior vein originated, the value is 470 sq. μ per nucleus. These figures indicate that the vein had not enlarged merely by



Text-fig. 7. Diameter of posterior vein of AV_7, plotted against time.

dilation of the capillary endothelium, and the observations tabulated in Table II confirm this for a number of vessels in different blastoderms. The values for area of endothelium per nucleus are all remarkably close, and we may conclude that even for the most rapid rates of increase in diameter of a vessel, the increase in the number of endothelial nuclei approximately keeps pace with the increase in surface area of the vessel wall.

Table II

Vessel	Average circumference (fixed)	Sq. μ per nucleus	Percentage of nuclei in division	Rate of increase from mitosis	Observed rate	Obs./Calc.
25 S, anterior region	135	405	3·6	—	—	—
	168	430	0·0	—	—	—
	185	340	4·0	—	—	—
27 S, anterior vein	625	520	5·0	—	—	—
AV_8. 36 S, anterior region	148	436	3·06	6·1	4·8	0·79
	235	435	1·0	2·0	9·9	5·0
	435	290*	6·6	13·0	39·6	3·05
AV_6. 35 S, anterior vein	576	415	2·23	4·56	18·2	4·0
RVA_1	710	508	2·3	4·6	4·3	0·94
RVA_2	495	350	2·04	4·1	3·5	0·85
AV_7. 37 S, left posterior vein, 6 hours after differentiation	505	303*	3·84	7·7	44·4	5·8
Capillary on opposite side	146	470	0·0	—	—	—

Rate of increase calculated as percentage increase per hour.

* The two lowest values for sq. μ per nucleus are those where percentage increase per hour is greatest.

The increase in diameter of the vessels therefore represents roughly the rate of increase in the number of endothelial nuclei. There are two possible ways in which new endothelial nuclei may arise, by division of existing endothelial nuclei, or by the entry of new cells into the endothelium from outside.

To decide which of these two alternatives is the correct one, we must calculate the rate of increase in area of the vessel wall which corresponds with the number of endothelial cells in division.

The relationship between the rate of increase in numbers of cells and the percentage of cells in division is given by the formula:

Percentage of cells seen in division = percentage rate of increase per hour × mitotic time in hours.

To apply this formula therefore, it is necessary to know the time occupied by the process of mitosis, and since cell division has not yet been followed within the living chick embryo, data obtained from chick tissue cultures must be used for this purpose. Strangeways (1922) states that "the time occupied for complete division of a cell, from the beginning of the prophase to the daughter nuclei being clearly seen, varied in thirteen cells, from 23 to 65 minutes, the average being 34 minutes". These results agree closely with the earlier work of Lewis and Levi.

For the purpose of the present work, an average mitotic time of half an hour has been assumed. It will probably not be possible to make a closer approximation until observations have been made on living intact chick embryos, and the variability of the time taken for cell divisions within the embryo estimated. In Table II therefore, the values for the percentage of nuclei seen in division has been multiplied by two to give the estimates for the rate of increase by mitosis. These are then compared with the observed rate of increase in the living state before fixation, and the last column gives the quotient obtained by dividing the calculated rate by the observed rate.

It can be seen that for the smaller rates of increase that were observed, namely from 3·5 to 4·8 per cent per hour, the calculated rate agrees closely with the observed, but for the higher observed rates of increase (19–44 per cent), these are from 4 to 5·8 times as high as the calculated values. This implies that this number of times more nuclei are being added to the vessel wall from outside than are being formed within the endothelium by division.

There are two main possibilities of error in these calculations. The first is in the estimation of the mitotic time, and the second is in the tacit assumption that the rate of cell division within the endothelium is constant not only during the half hour occupied by one division, but also over the whole preceding period, when the rate of increase in the diameter of the vessel was observed for comparison with the rate calculated from mitosis. However, even if we allow a generous margin for these two sources of error, it is hardly possible to escape the conclusion that nuclei enter the endothelium from outside when a vessel increases in diameter rapidly. The most likely source of these nuclei is the mesenchyme surrounding the vessel, and a histological study was made to find whether there was evidence of such a relationship between endothelium and mesenchyme.

In counting endothelial nuclei in sections, it is extremely difficult to decide exactly which nuclei are endothelial, and which belong to the mesenchyme bordering the vessel. Plate II, fig. 7 is a photomicrograph of a section through the posterior vein of AV_7, to illustrate this point. In counting, the line of the endothelial membrane was used as the criterion; those nuclei which lie within this line, or are cut by it, are counted as endothelial, and those outside it as mesenchymal. It is clear that every intermediate condition exists between those which definitely belong to one group and those which belong to the other. This fact provides substantial indirect evidence that endothelial cells are supplied from the mesenchyme, but this view cannot be regarded as proved until the entry of mesenchyme cells into the endothelium has been observed in the living tissue.

In the previous paper of the series (Hughes, 1935), a hypothesis of arterial growth is given, based on cell division within the endothelium. The relationship of this hypothesis to the findings described in this section must now be discussed. In the first place, the greatest observed rate of enlargement of an artery which we have noted in this work is 5 per cent of the original diameter

per hour, whereas the greatest observed rate for a vein is 40 per cent. In Table II, the rates of increase per hour by mitosis have been calculated for two arteries, and are 4·6 and 4·1 per cent per hour, the corresponding observed rates being 4·3 and 3·5 per cent respectively. Their rates of increase are thus fully accounted for by mitosis. It may therefore be that vessel walls which increase comparatively slowly in area do so entirely by mitosis, possibly in the way suggested in the earlier paper, and that only the more rapid rates of increase depend on the incorporation of the mesenchyme.

(2) *Increase in vessel length*

The proximal part of the posterior vein does not increase very greatly in length. In the anterior region of the area vasculosa, there are vessels which are changing in diameter and increasing in length at the same time. To examine the effects on the growth of endothelium in vessels which were simultaneously changing in both length and diameter, the anterior region of AV_8 was studied (Text-fig. 8 and Plate III).

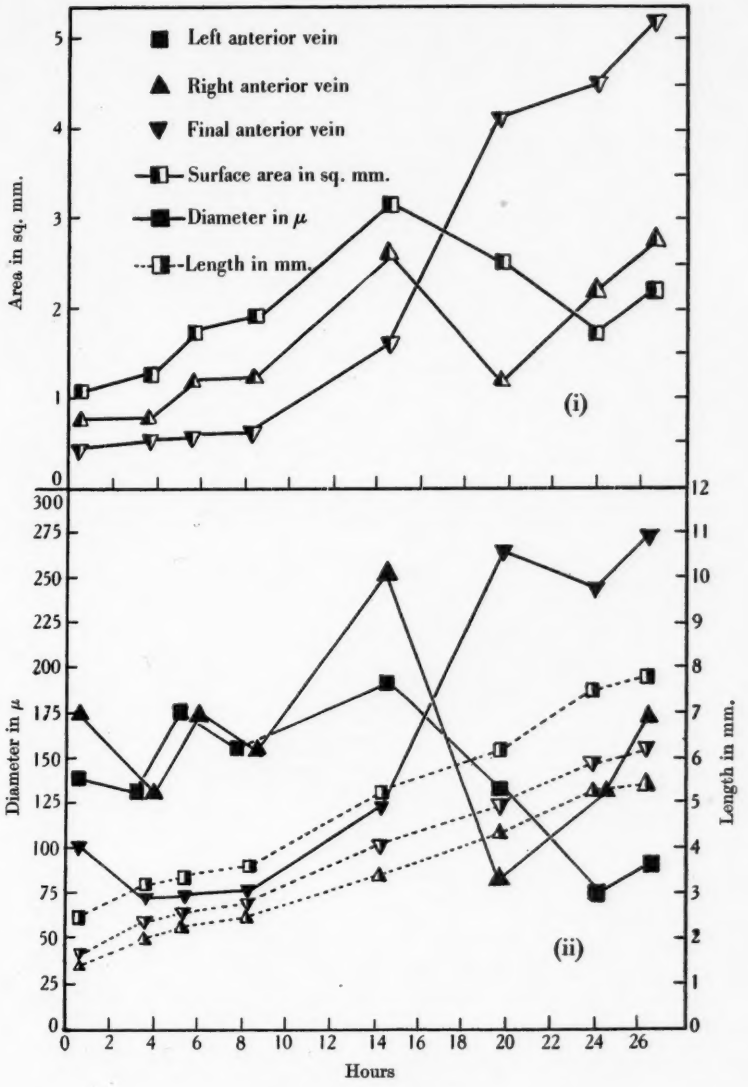
In AV_8, the final anterior vein was formed not from either of the two original anterior veins, but from a capillary in between them.

Text-fig. 8 shows graphically the changes in length, diameter and area of wall of the two original anterior veins and of the vessels which became the single anterior vein. In Table II the rates of mitosis in the endothelium of these three vessels are given, and also their corresponding rates of increase in area by cell division. The values obtained for these latter rates range from 2 to 13 per cent of the original diameter per hour; the observed rates on increase in length of the same vessels are 8·5, 9·7 and 12 per cent. It is therefore doubtful if the mitotic rates of these peripheral vessels are sufficiently high to keep pace with the actual growth of the area vasculosa, for the vessel with the highest calculated rate of increase by mitosis, namely 13 per cent, is the one which steadily increases in diameter, and in which the endothelium actually increases in area at the rate of 39·6 per cent per hour. Conversion of mesenchyme into endothelium therefore may well be part of the normal process of extension of the vascular area, apart from the influence of the circulation on the diameter of the vessels.

THOMA'S HYPOTHESIS IN RELATION TO THESE RESULTS

The results described in the present paper show that there is a general correlation between marginal velocity and rate of change in diameter for vessels of the area vasculosa from the second to the fourth day of incubation. Since a relationship must exist between marginal velocity and rate of flow, there must also be a correlation between rate of change of diameter and rate of flow.

This correlation is not a simple mathematical relationship for the following reasons. The relationship between marginal velocity and rate of flow depends



Text-fig. 8. Growth of three vessels in the anterior part of AV_8. The data are derived from the photographs shown in Plate III. (ii) gives the diameter and length of the three vessels marked in Plate III, and (i) gives the surface area of these vessels, obtained from the data in (ii).

on the diameter; the relationship between marginal velocity and rate of change in diameter depends also on the diameter. A given marginal velocity does not produce the same rate of change in diameter at two different diameters. The rate of change in diameter depends also on the amount of change in diameter during the preceding few hours.

Thoma's explanation of his hypothesis was that the rate of flow of the blood determined the rate of supply of nutritive substances to the vessel wall from the blood stream, and that this rate of supply controlled the rate of growth of the wall. Arguments against this hypothesis were discussed in the preceding paper (Hughes, 1935). To them we can now add that growth in the area of vessel wall is only one way in which an embryonic vessel increases in diameter. Cells from the surrounding mesenchyme are incorporated into a vessel wall which increases rapidly in area, a process to which Thoma's nutritive hypothesis does not apply.

With these reservations, the present work confirms the hypothesis as applied to the early embryonic vessels of the chick area vasculosa.

SUMMARY OF RESULTS

1. A description is given of a method by means of which a chick embryo, growing in an opened egg, can be observed continuously with an indirect microscope over a period of several days.

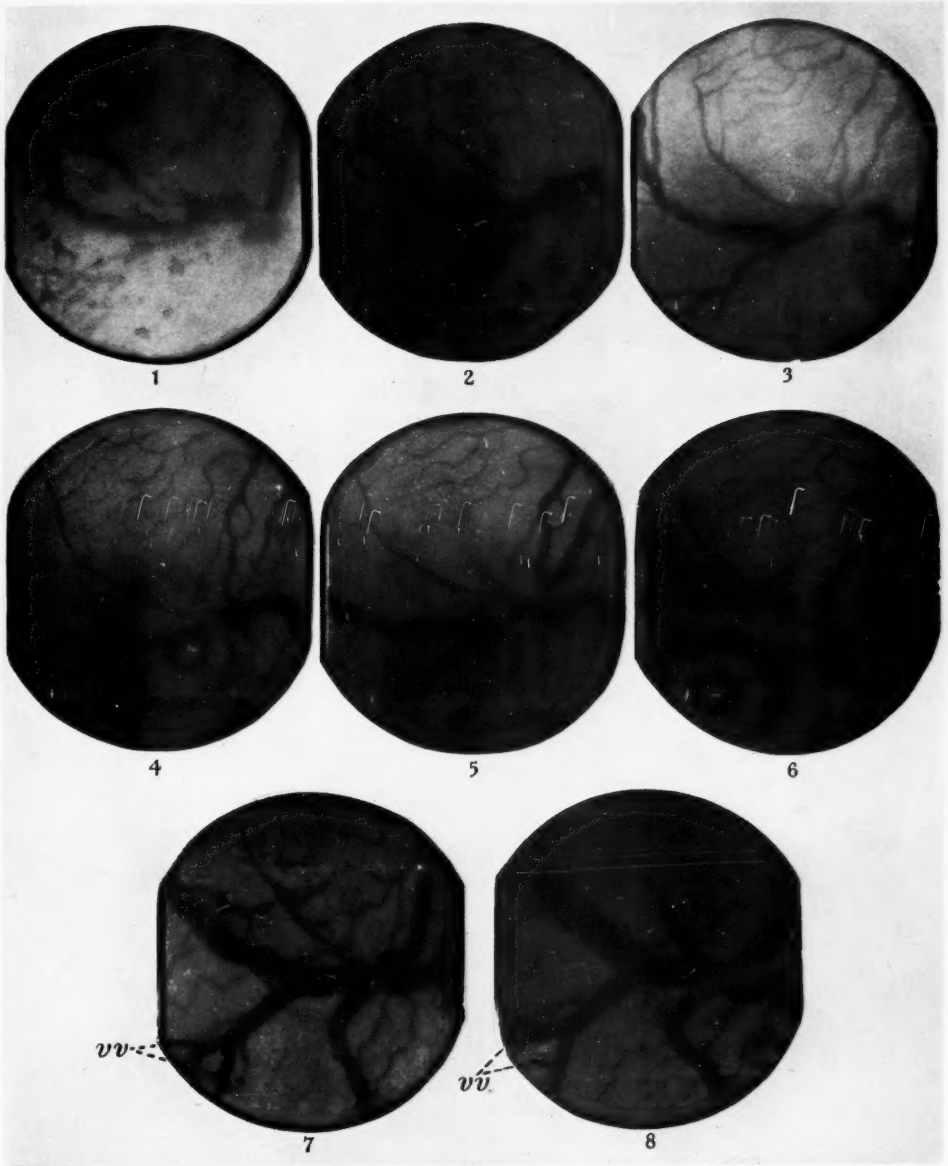
2. During the third and fourth days of incubation, the maximum rate of increase in diameter of the arteries of the area vasculosa is 5 per cent per hour. The maximum velocity of the superficial corpuscles in the arteries is about 1·2 mm. per sec. During the first 10 hours of the formation of the posterior vein, the rate of increase per hour may be as much as 40 per cent per hour, and the maximum velocity of the superficial corpuscles is about 4 mm. per sec.

3. Increase in number of endothelial nuclei is found to keep pace with increase in diameter of the vessels, and no increase in cell size was found in the most rapidly enlarging veins.

4. It is found that the mitotic rates in the endothelium of the arteries are sufficient to account for their rates of increase in diameter. In veins increasing rapidly in diameter, cell division may account for only one-fifth of the actual increase in number of endothelial cells. The remaining four-fifths of the cells appear to be derived from the mesenchyme bordering the vessel.

5. The growth of endothelium in the outward extension of the area vasculosa probably takes place by the conversion of mesenchyme into endothelium, as well as by cell division within the vessel walls.

6. The bearing of these results on Thoma's hypothesis of vessel growth is discussed.



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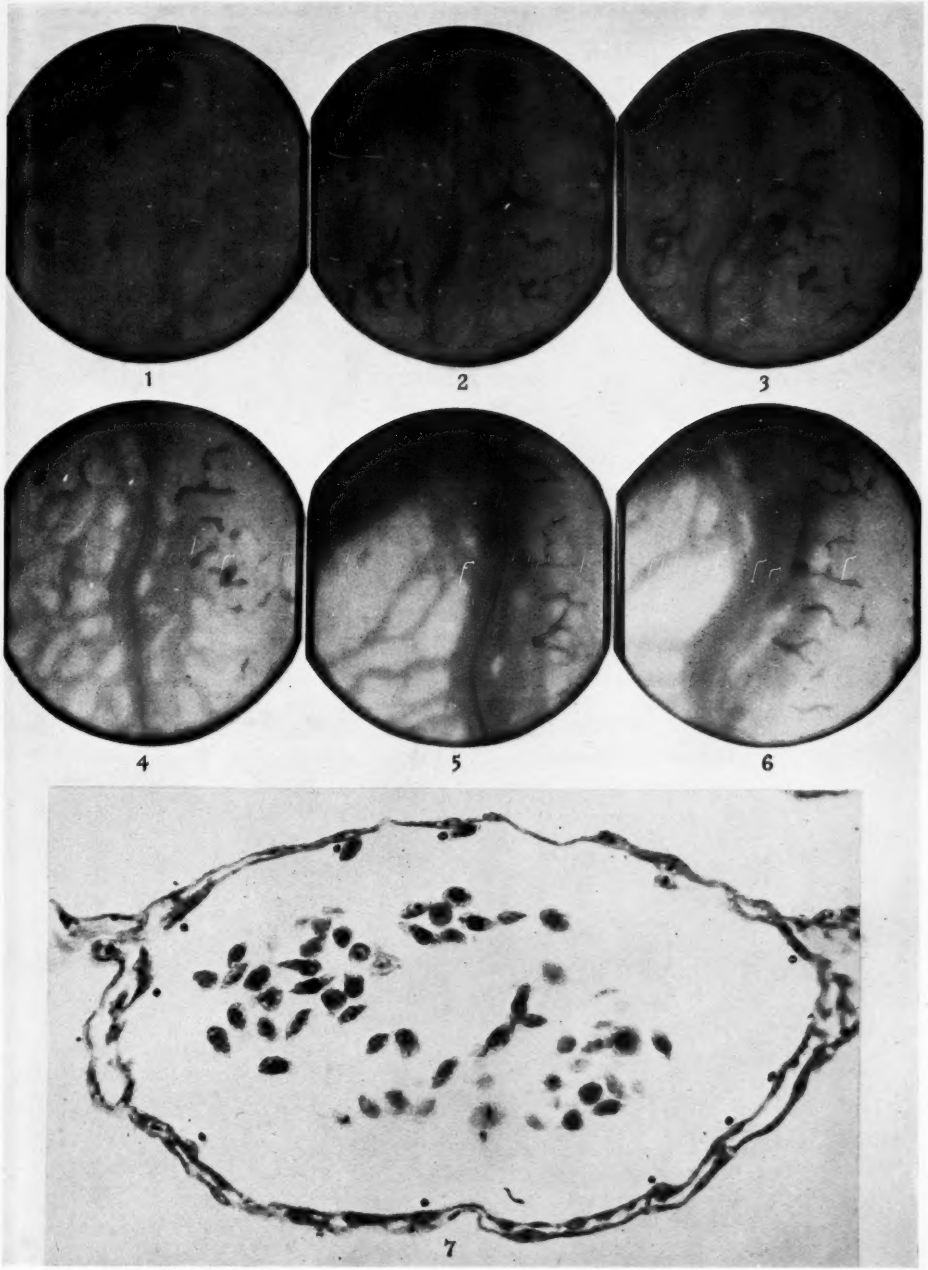

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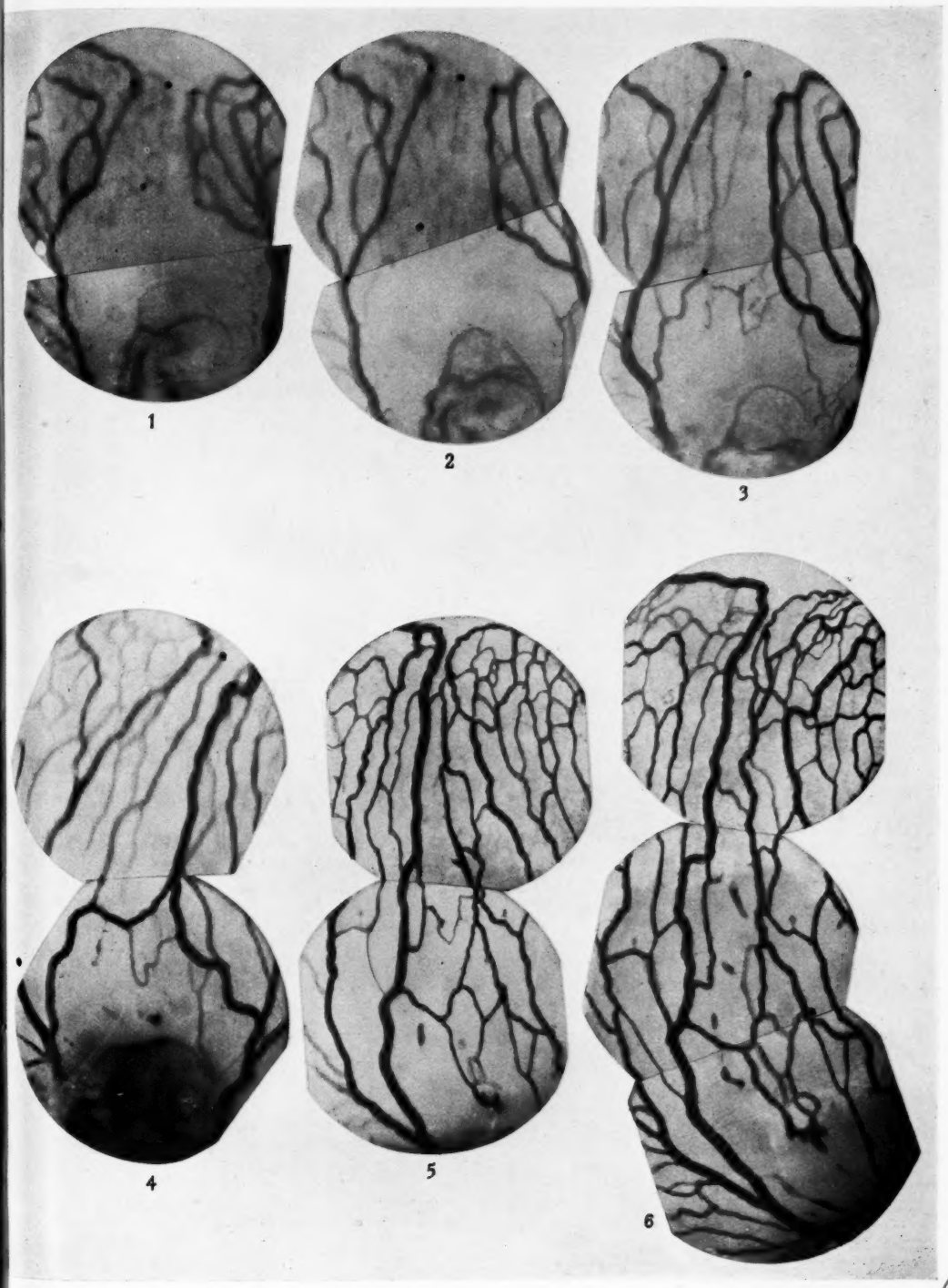

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ACKNOWLEDGEMENTS

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EXPLANATION OF PLATES I–III

PLATE I

Photographs of the left vitelline artery and its surroundings of AV_1. The diameters and marginal velocity of the three branches of the artery and of the posterior vein are plotted in Text-fig. 1. The vessels marked *vv* are part of the lateral vein system. × 14. Time of photographs: fig. 1, 10 a.m.; fig. 2, after 4½ hours; fig. 3, after 7½ hours; fig. 4, after 9 hours; fig. 5, after 11 hours; fig. 6, after 13 hours; fig. 7, after 19½ hours; fig. 8, after 24 hours.

PLATE II

Figs. 1–6. Photographs of the posterior vein of AV_7, in the region immediately posterior to the vitelline artery. × 41. Time of photographs: fig. 1, 3 p.m.; fig. 2, after 2¼ hours; fig. 3, after 3¼ hours; fig. 4, after 5¼ hours; fig. 5, after 7½ hours; fig. 6, after 9½ hours.

Fig. 7. Section through the posterior vein AV_7, to illustrate the difficulty in distinguishing between endothelial and mesenchymal nuclei bordering the vessel. A dot is placed opposite the eleven nuclei which were counted as endothelial in this section. × 470.

PLATE III

Photographs of the anterior region of AV_8, from which the data in Text-fig. 8 were obtained by measuring diameter and length of the left and right anterior veins, and of the vessels between them which became the final anterior vein. The length of the vessels was measured between the points marked. × 14. Time of photographs: fig. 1, 3·25 p.m.; fig. 2, after 5 hours; fig. 3, after 8 hours; fig. 4, after 14 hours; fig. 5, after 19 hours; fig. 6, after 26 hours.

THE RELATION BETWEEN THE SIZE OF THE ARTERY AND THE CAPILLARY BED IN THE EMBRYO

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THE work of Mall (5), Evans (2), Woollard (7), Hughes (3,4), and others has established that there is a stage in the embryo after the formation of the capillary bed when definitive arterial stems arise by enlargement and fusion in part of the capillary bed, and coincident atrophy in other parts. The emergence of these definitive stems is gradual and intermediate stages can be discovered where the fusion of the capillaries is not yet complete. Observations of close stages of injected preparations make it certain that this mode of development is an accurate account of what happens.

Clearly it is to this stage of the erection of definitive stems that Thoma's laws apply, the earlier phase of capillary elaboration, it is generally agreed, being an angioblastic response. Thoma's laws have often been stated, but perhaps they may be repeated here in the form quoted by Mall (5). The first law is that increase in the size of the lumen of the vessel, or, what is the same thing, the increase of the surface of the vessel wall, depends upon the rate of the blood current. The second law states that the growth in thickness of the vessel wall is dependent upon the tension. Further, the tension of the wall is dependent on the diameter of the lumen of the vessels and upon the blood pressure. The third law, which is the most contentious one, states that an increase of blood pressure in an area leads to the new formation of capillaries.

The background of these laws has been discussed several times, most recently by Hughes (4,5), and it seems clear that endothelium can grow by its own intrinsic properties. It is not the blood flow through them that induces them to grow; this merely keeps up the regular pattern. Hughes and others have, however, pointed out that this conclusion is not necessarily in conflict with Thoma's third law, since it is quite possible that in a particular field one of the factors inducing new capillary formation might be an increase in the blood pressure of the particular region concerned. This third law has been invoked by Clark *et al* (1), for instance, to explain the growth of the new capillaries which they observed in their experiments on the rabbit's ear.

It is believed that the facts drawn from pathology and assembled by Thoma himself have given an assured basis to the second of these laws. The most spectacular example of the second law is found in the arterial-like changes

which a vein undergoes when it is subjected to the forces operating upon an arterial wall by introducing it into an arterial stem.

Thoma's work on the area vasculosa of the chick has long been regarded as the principal support of the first of these laws, but it is true that for the most part in the embryological field this law has been based on its interpretative value rather than supported by quantitative investigation. Hughes in his latest work (which we have had the privilege of seeing in manuscript) has attempted to make good this defect by a very extensive quantitative investigation on the blood vessels of the developing chick. He has taken as his source of observations the marginal velocity of the corpuscles wherever measurable in arteries and veins. Owing to the very great difficulties encountered in plotting vessel size against such velocities as can be seen and measured, there is a considerable degree of inconstancy in the results obtained.

PRESENT INVESTIGATIONS

It occurred to us that in fixed injected specimens it might not only be possible to make more accurate measurements, but also that it would be much easier to measure the diameter of the entering and leaving blood vessels and the area of the capillary bed which they supplied. This implies of course the assumption that the amount of blood delivered to the capillary area would be proportional to its area and would also be proportional to the cross-sectional area of the vessel of supply.

There were available to us a number of pig embryos which had been injected while still living with indian ink. These we had used previously for the study of the development of the blood vessels of the forelimb. The injected specimens had been fixed in formalin and cleared through oil of wintergreen. Out of the material we selected a number in which the limbs had been detached and in which the filling of the vessels appeared to be complete.

Under the microscope, by means of camera lucida tracing, it was possible to measure the diameter of the entering vessels and the area covered by the injected capillaries. Areas were measured either by using squared paper or by means of a planimeter. The diameter of the entering blood vessel was obtained in the same way.

The graph shown as Fig. 1 is the outcome of these measurements upon arteries and capillary beds. It will be seen that at the stage when the arteries are becoming differentiated from the capillary bed the graph shows a close approximation to a straight line. These measurements cannot claim any great precision, for the pressure (blowing by the mouth) at which the injections were made might differ somewhat, the resistance to distension perhaps would vary in the different embryos, and the state of contraction or dilatation of the capillary bed might not be the same. These considerations can be used either to suggest that the agreement between vessel size and capillary bed is greater than appears, or that the relationship is more apparent than real. It is also to be

observed that the graph shows a change from the linear relation at the beginning and towards the end of the instances plotted. The earlier curve occurs presumably because then our examples fall within the period of the angioblastic response which is forming the capillary bed, and the second later curve because we now reach toward the more stable relation that arises as the rate of growth declines. The curve hereafter will approach more and more to the horizontal as growth becomes slower and slower.

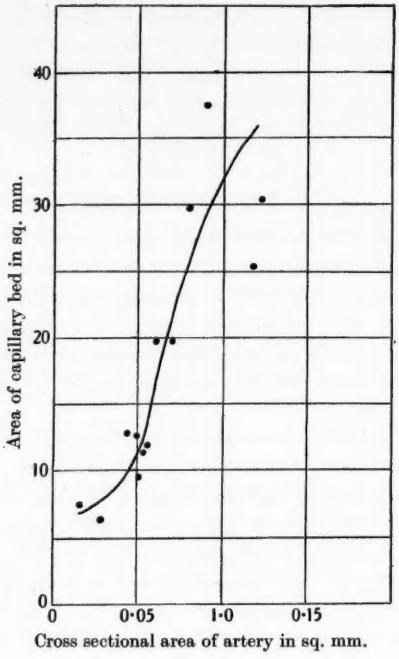

Fig. 1

The illustrations which accompanied our earlier paper (7) could be used also for the construction of a graph, since in each the capillary bed and the size of the artery had been accurately drawn. Since, however, they had not been all drawn at the same degree of magnification it was necessary, in order to make a graph, to plot the ratio of the size of the artery to the area of the capillary bed against the length of the embryo. This gave us a straight line, but the cases illustrated are too few to justify publishing the result.

In these computations we have measured the area of the capillary bed rather than its volume. Obviously it would have been more satisfactory to have used this latter measurement. In practice the difficulties of getting an accurate measure of cubic capacity would have been so great that many new

errors would have been introduced. The measurement of the area was really of more value.

The measurements obtained for the veins were unsatisfactory. In our preparations the diameters of the veins change frequently and irregularly along their course. Further, sometimes a vein which may have attained a relatively considerable volume along its course might relapse into a capillary network again in the vicinity of the body wall. However, when selecting older specimens in which these difficulties did not occur, it can be said that there is some correspondence between vein size and the area of the capillary bed.

After taking into account the various sources of error in these measurements, and after the admission that they measure only indirectly the rate of blood flow into a capillary bed, we believe that they help us to justify the validity of Thoma's law in regard to the development of blood vessels in the embryo. The matter seems to us of some importance. Frequently the development of blood vessels, for example those of the lower extremity, are described as exhibiting a series of changes wherein the pattern of large definitive stems changes in a manner for which there seems to be neither functional nor morphological reason. If one accepts Thoma's laws, or even only the first one of them, one must feel some scepticism about these apparently inexplicable changes.

The evidence behind these laws of Thoma is now considerable. There is the great work of Mall (5) on the structural unit of liver, wherein he showed by numerous measurements how Thoma's laws hold throughout development, and serve to ensure the functional value of the adult arrangements. Upon the development of veins the laws of Thoma seem much more doubtful. Even in the earlier stages of development the correspondence, measured as described above, between size of vein and capillary bed is not close and in the adult the dimensions of the venous wall, the size of the vein, and the pressure within it have only the most general relationship to one another.

SUMMARY

1. Using the injected blood vessels of pig embryos at different ages the size of the artery and the area of the capillary bed supplied in the limb have been measured.

2. Plotting these against each other an approximate straight line has been obtained within certain limits.

3. Within these limits it has therefore been concluded that the size of the artery depends on the amount of blood passing unit cross-sectional area in unit time, or the rate of the flow of blood which is Thoma's first law.

4. The same observations applied to veins at the same stage of development exhibit only very slight agreement.

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THE DEVELOPMENT OF THE CEREBROSPINAL FLUID SPACES AND CHOROID PLEXUSES IN THE CHICK

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I. INTRODUCTION

The work described in this paper is part of a study comprising the comparative morphology and development of the choroid plexuses, the meninges and the meningeal spaces. Chick embryos of accurately determined age were investigated, utilizing a similar method to that adopted by L. H. Weed in his classical studies on pig and human embryos.

II. SURVEY OF LITERATURE

(*a*) *The development of the cerebrospinal fluid spaces in the Mammalia*

Two workers, L. H. Weed (1917) and J. J. Keegan (1917), have investigated the development of the cerebrospinal fluid spaces by the method of injecting a true solution into the central nervous system of the living embryo. Weed's comprehensive monograph appeared in 1917, and very shortly after Keegan published a very brief report of his own investigations. In a personal communication to us, Dr Keegan stated that in view of Weed's earlier publica-

tion of almost identical work, he felt it was unnecessary to make a further detailed report of his own findings. In the light of the present investigation, however, Dr Keegan's paper, brief though it was, proved to be of great interest.

Weed (1917) investigated the condition of the cerebrospinal fluid spaces in pig embryos at different stages of development. He replaced the fluid within the central nervous system by a 1 per cent double solution of potassium ferrocyanide and iron ammonium citrate. Fixation of the embryo in an acid medium secured precipitation of Prussian blue (ferric ferrocyanide) *in situ* and thus revealed the path taken by the injection solution. He records that the first extra-ventricular spread of the injected solution occurred in pig embryos of 14 mm. length. Further, he found that the injected fluid made its escape into the surrounding mesenchymal tissue through two differentiated areas, one anterior and the other posterior, in the roof of the fourth ventricle. Weed terms these areas the *area membranacea superior* and the *area membranacea inferior* respectively, and states that both "differentiate at a slightly earlier period than that at which they function actively" (1917, p. 107). The cells of these areas are distinguishable by their low stature and narrow, oval or slightly elongated nuclei.

The area membranacea superior is first differentiated in embryos of 11 mm. and reaches its maximum development at the 18 mm. stage. The fully differentiated form is maintained until about the 20 mm. stage, when the area commences a gradual regression, and disappears completely in embryos of 33 mm. Its disappearance is brought about by the encroachment of the developing cerebellum on the roof of the fourth ventricle, and by the formation of the choroid plexus.

The inferior area shows the first signs of differentiation in embryos of 15 mm.; it develops but slowly until the 18 mm. stage is reached, then differentiates extremely rapidly. Unlike the superior area, it persists and "gradually occupies the major portion of the velum choroidea inferior" (Weed, 1917, p. 107).

The first escape of fluid from the spaces of the central nervous system in the 14 mm. embryos was found to be coincident with the first indication of tufting of the developing choroid plexus of the fourth ventricle. A further marked extra-ventricular extension of the injected solution occurred in embryos of 19 mm., and this was associated with the development of the choroid plexuses of the third and lateral ventricles at this stage. Furthermore, these plexuses were only definitely differentiated in embryos of 23 mm., and at this stage a complete periaxial spread occurred. From these observations Weed concludes that "there seems to be a very definite relationship between the developing choroid plexuses and the periaxial spread of the embryonic cerebrospinal fluid" (1917, p. 93).

Weed has also identified and described similar areas of differentiation in the roof of the fourth ventricle of human foetuses at different stages of development, and the sequence of events resembles that found in pig embryos.

The presence of an area membranacea superior is also recorded in the embryos of chick, rabbit, sheep and cat, but no mention is made of an inferior area in these types.

Keegan (1917) injected a 1 per cent double solution of potassium ferrocyanide and iron ammonium citrate into the brain cavities of living rabbit and chick embryos at various stages of development, and secured precipitation of Prussian blue granules by fixation in an acid medium.

He records in both types the presence of two thin oval areas, one anterior and the other posterior to the choroid plexus, in the roof of the fourth ventricle. Each area was associated with a dense collection of protein coagula on its ventricular surface. In all early embryos injected with the double solution, a condensation of precipitated Prussian blue granules was also found below these areas.

It is further reported that "injection with the double solution in rabbit embryos up to the age of 17 days showed no extra-ventricular spread of the fluid, which was rather surprising in view of the fact that the choroid plexus is quite well developed at this age" (Keegan, 1917, p. 379). On the other hand, when a solution of ferric ammonium citrate alone was injected, extra-ventricular spread of the fluid occurred in the region of the fourth ventricle *before* the development of the choroid plexus, and there was only a slight condensation of blue granules below the thin areas of this roof. "After the development of the choroid plexus there was a very rapid absorption into the circulation" (Keegan, 1917, p. 380).

Similarly, in chick embryos injected with the double solution, no extra-ventricular spread of the fluid had occurred, even in 9 days' embryos, although the choroid plexus was present at this stage. Again, when the citrate solution alone was injected, extra-ventricular spread of the fluid invariably occurred, "and after the development of the choroid plexus (sixth to seventh day) very rapidly entered the circulation. At the seven day stage, a considerable portion remained within the ventricle and as a very evident blue coloration in the mesenchymal tissue over the rhombencephalon and mesencephalon.... In the eight and nine day chick the escape of the citrate solution was even more rapid, practically all leaving the ventricle within 10–20 min." (Keegan, 1917, p. 380).

Keegan infers therefore that "this membranous area of the roof of the fourth ventricle is non-permeable to the double solution in the early embryo stages while it is permeable to the citrate solution; that a slight escape of the cerebrospinal fluid occurs before the development of the choroid plexus; and that the collection of protein coagula and Prussian blue granules in contact with the inner surface of the membranous area represents a dialysis phenomenon of this semi-permeable membrane" (Keegan, 1917, p. 380).

No details are given of the histological features of the membranous areas in the roof of the fourth ventricle, nor of the exact stages at which these areas make their appearance; neither is any mention made of their ultimate fate. Moreover, Keegan in his use of the term "choroid plexuses" does not clearly

distinguish between those of the lateral and third ventricles and that of the fourth ventricle—a distinction which is shown to be important in the light of the present investigation.

(*b*) *The avian meninges*

Cuvier (1809) and Owen (1868) stated that three meninges are present in birds, a pia mater, arachnoid and dura mater, essentially similar to those found in the Mammalia and bearing the same relation to each other.

Sterzi (1902), on the other hand, states that only two meninges are present in birds, an outer, thin, non-vascular dura mater, and an inner, secondary meninx, these being comparable to the two meninges found in the reptilia. The secondary meninx further consists of three layers, a thin, outermost endothelial layer, a middle vascular layer of loose texture, and an inner layer immediately adjacent to the cord. This structure of the secondary meninx shows an advance on the reptilian condition, where it is a thin single layer, and foreshadows the pia and arachnoid of the Mammalia.

Streeter (1904), investigating the condition of the meninges in the ostrich, asserts that three membranes are present, similar to those found in the Mammalia.

The first detailed description of the development of the avian meninges appears to be that given by Farrar in 1906. In his description of the development of the pia-arachnoid membrane in the chick, Farrar designates the general mesenchymal tissue surrounding the embryonic nervous system as the arachnoid sheath or mesh, and states that the pia-arachnoid sheath arises from the cells of the arachnoid mesh immediately adjacent to the nervous system. The pia-arachnoid develops as a "single membrane consisting of a loose reticulum, the trabeculae of which are formed by the branching and anastomosing processes of connective tissue cells. At the outer and inner borders these cells tend to arrange themselves horizontally to form limiting membranes" (Farrar, 1906, p. 297). Blood channels make their appearance within the developing pia-arachnoid and increase in number to such an extent as to form an almost uninterrupted chain of vascular spaces within this zone. These blood channels are essentially similar to those found in the general arachnoid mesh. While the pia-arachnoid is developing from the inner zone of the arachnoid mesh, a second outer non-vascular zone of condensation makes its appearance, separated from the pia-arachnoid by an intermediate region of undifferentiated mesenchyme. In this outer zone, "the connective tissue elements are assuming elongated forms and crowding together with long axes parallel, giving rise to a very close mesh with long but extremely narrow spaces, in contradistinction to the loose, irregular reticulum of the pia-arachnoid" (Farrar, 1906, p. 297). This outer zone is the primitive dura and is clearly demarcated from the pia-arachnoid. According to Farrar, therefore, two meninges are present in the chick, an outer dura and an inner pia-arachnoid; "in the thickness of the latter,

however, no separating line can be drawn subdividing it into two membranes" (1906, p. 297).

No details are given of the stages at which these developmental changes occur.

Lillie (1908), in his description of the developing brain of the chick, records that up to the eighth day the roof of the fourth ventricle retains its thin epithelial character, whilst the floor and sides thicken considerably, but no statement is made regarding the existence of any thin differentiated roof areas.

Hansen-Pruss (1923) maintains that three distinct meninges are present in birds. Moreover, he demonstrated the presence of cerebrospinal fluid in the loosely trabeculated subarachnoid space and succeeded in injecting into this space India ink, and also a double solution of potassium ferrocyanide and iron ammonium citrate. Further, he found that the injected fluids "extended caudally, surrounding the spinal cord, to the upper pole of the sinus rhomboidalis" (p. 206). Cephalad, the injection fluids were traced "over both hemispheres and optic lobes as far forward as the sinus frontalis and down on the median and lateral sides" (p. 206).

Ariëns-Kappers (1929), and Ariëns-Kappers *et al.* (1936) state that in birds two meninges are present, an outer dura and an inner meninx, also termed the secondary meninx, and in which small arachnoid spaces make their appearance.

Thus, according to Cuvier (1809), Owen (1868), Streeter (1904) and Hansen-Pruss (1923), three meninges exist in birds. Sterzi (1902), Farrar (1906), Ariëns-Kappers (1929), and Ariëns-Kappers *et al.* (1936) on the other hand, maintain that only two such membranes are present.

III. MATERIAL

The present account is based on observations of six series of chick embryos, which for purposes of convenience are referred to as series A, B, C, D, E and F respectively.

The eggs of each series were incubated at a temperature of 38–40° C., care being taken that these limits were not exceeded.

IV. METHODS OF INVESTIGATION

Immediately prior to injection the embryo was removed from the egg together with a portion of the extra-embryonic yolk-sac, and placed in Pannett and Compton's saline solution, warmed to a temperature of 38–40° C. This solution was prepared in the following way from two stock solutions:

Stock solution A:

Sodium chloride (NaCl)	...	...	12·11 g.
Potassium chloride (KCl)	...	...	1·55 g.
Calcium chloride ($CaCl_2$)	...	...	0·77 g.
Magnesium chloride ($MgCl_2.6H_2O$)	...		1·27 g.

Distilled water to make 100 c.c. of solution.

Stock solution B:

Prepared from the following solutions:

(1) Sodium dihydrogen phosphate ($NaH_2PO_4.12H_2O$): distilled water to make 100 c.c. of solution ... 0·2 g.

(2) Disodium hydrogen phosphate ($Na_2HPO_4.12H_2O$): distilled water to make 100 c.c. of solution ... 0·52 g.

These solutions are mixed in the following proportions:

Solution (1) ... 5 c.c.

Solution (2) ... 55 c.c.

and are then autoclaved.

The final solution consists of 1 c.c. of stock solution A and 1·5 c.c. of stock solution B in 22·5 c.c. of distilled water.

The embryo was exposed by careful removal of the chorion and amnion, and was kept in the warm saline solution throughout the injection.

For the purpose of injection two types of solutions were used, a double solution of potassium ferrocyanide and iron ammonium citrate, and a solution of iron ammonium citrate alone. It was found by experiment on the red blood corpuscles of the hen that a solution of 0·8 per cent concentration[1] was for practical purposes isotonic with the body fluids of the embryo, and this concentration was used throughout the investigation.

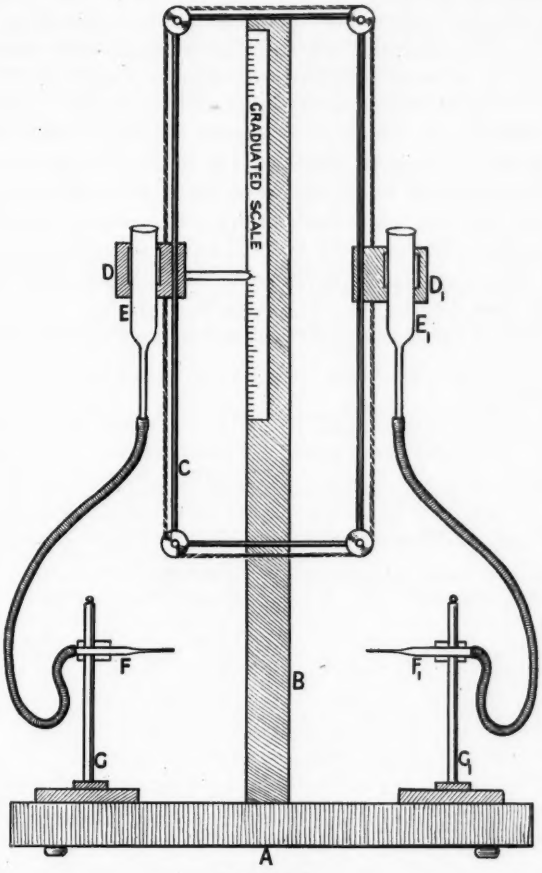
During the present investigation injections were made either into the central nervous system or into the vascular system.

Two methods of injecting the fluid were employed. The first method was by means of a short glass tube of narrow bore drawn out into a fine capillary point at one end, and provided with a small rubber bulb at the other. In the case of injection into the central nervous system, a small quantity of fluid was injected under very slight pressure into the caudal end of the central canal of the spinal cord. Undue increase of intra-cerebral pressure was avoided by simultaneous withdrawal of any fluid contained within the central nervous system. This was effected by inserting a similar capillary needle into the lateral ventricle of either cerebral hemisphere. In the case of injection into the vascular system, a minute quantity of fluid was injected into the left ventricle of the heart under very slight pressure. After the dye had passed from the ventricle into the aorta, the injection was repeated and this procedure was continued until the blood vessels of the head region were seen to contain the injected dye.

The second method of injection was replacement of the fluid within the central nervous system by the injection solution, using the apparatus shown in Text-fig. 1. This consisted of a flat wooden base (*A*) supporting an upright rod (*B*), to which was affixed a graduated scale indicating the pressure in millimetres of water under which the injection was made. The upright rod also carried a rectangular brass frame (*C*), provided with a small pulley at each of

[1] In the case of the double solution, 0·4 g. of each salt were dissolved in a 100 c.c. of solution; in the case of the single solution 0·8 g. of iron ammonium citrate were dissolved in a 100 c.c. of solution.

its four corners. The longer bars of this frame supported two spring clamps (*D*, D_1) fitted with rollers in order to allow of easy movement along the bars. A length of Egyptian cotton, made taut around the pulleys, was passed through the clamps, and thus their relative position could be adjusted by movement of the cotton. Each clamp held a glass reservoir (*E*, E_1) connected to a capillary



Text-fig. 1.

needle (*F*, F_1) by a length of rubber tubing. In addition, the clamp *D* carried a pointer which moved up and down the graduated scale, according to the position of the clamp. The capillary needles were fixed into adjustable metal holders (*G*, G_1) which provided free movement in all planes.

The reservoir *E* was filled with the injection fluid until drops appeared at the tip of the needle. The reservoir E_1 was filled with 0·75 per cent saline solution

in the same manner. The flow of the fluid from each needle could be completely arrested by means of a clip placed on the rubber tubing at a short distance behind the needles.

The advantage of this apparatus is twofold—the reservoirs are firmly supported but can still be moved freely up and down, and the pressure under which the injection is made is automatically recorded.

The embryo to be injected was supported on a block placed on the stand *A*. The reservoirs were filled with the injection fluid and saline solution to the same level. The needles were adjusted to a suitable position and then inserted into the embryo, needle *F* into the caudal end of the central canal of the spinal cord and needle F_1 into the lateral ventricle of either cerebral hemisphere. When both needles were in position the clips were removed from the rubber tubing and the reservoir *E* was slowly raised, while the reservoir E_1 was lowered simultaneously. As fluid was withdrawn from the central nervous system into the needle F_1, it was automatically replaced by the injection solution from the needle *F*. It was found that complete replacement was effected at a pressure equivalent to a column of water 70–75 mm. in height.

The methods of treatment of the different series of embryos are tabulated below.

Series	Age limit of series days	System injected	Injection fluid used
A	3–9	Central nervous system	Double solution of potassium ferrocyanide and iron ammonium citrate
B	3–9	Central nervous system	Double solution of potassium ferrocyanide and iron ammonium citrate
C	4½–9	Central nervous system	Double solution of potassium ferrocyanide and iron ammonium citrate
D	3½–9	Central nervous system	Solution of iron ammonium citrate
E	5–9	Vascular system	Double solution of potassium ferrocyanide and iron ammonium citrate
F	5–9	Vascular system	Solution of iron ammonium citrate

The embryos were injected between the age limits stated at intervals of approximately 12 hours. In each series at least two embryos were injected at each stage of development. After each injection the embryo, still in the saline solution, was kept alive in an incubator at 38–40° C. to allow of the spread of the injection fluid, the embryonic heart-beat serving as the criterion of survival. It was found that ½–¾ hour in the case of the smaller embryos, and 1–1½ hours in the larger, were needed for complete spread.

Embryos injected with the double solution were then placed in a solution containing 10 per cent formaldehyde and 1 per cent hydrochloric acid. This caused precipitation of Prussian blue (ferric-ferrocyanide) and partial fixation of the embryo. Specimens injected with iron ammonium citrate alone were placed in a similar acid formaldehyde solution, to which some potassium ferrocyanide solution had been added. This also caused precipitation of Prussian blue and partial fixation. The time required for precipitation was ½–1 hour, according to the size of the embryo.

The members of series A were then left in Bouin's fluid overnight to complete fixation, but this resulted in a yellow colouration which obscured the external outline of the course of the injection fluid. Consequently, the embryos of the remaining series were fixed by allowing them to remain in the acidic formaldehyde solution overnight. Thus, the course of the injection fluid remained clearly visible, and the subsequent histological picture was in no way altered.

After fixation the embryos were dehydrated in a series of alcohol solutions, increasing from 5–100 per cent concentrations by 10 per cent gradations. They were then cleared in xylol or cedarwood oil,[1] embedded in paraffin wax (52 or 56° C. M.P. according to season), and serially sectioned at a thickness of 4 μ, some in the longitudinal and some in the transverse plane. Ehrlich's haematoxylin and eosin were found to be the most suitable stains, and were used throughout the investigation.

V. DESCRIPTION OF THE BRAIN AND MENINGES OF THE CHICK AT DIFFERENT STAGES OF DEVELOPMENT, WITH PARTICULAR REFERENCE TO THE STRUCTURE AND PERMEABILITY OF THE ROOF OF THE FOURTH VENTRICLE

(*a*) *Results of injections of true solutions into the central nervous system*

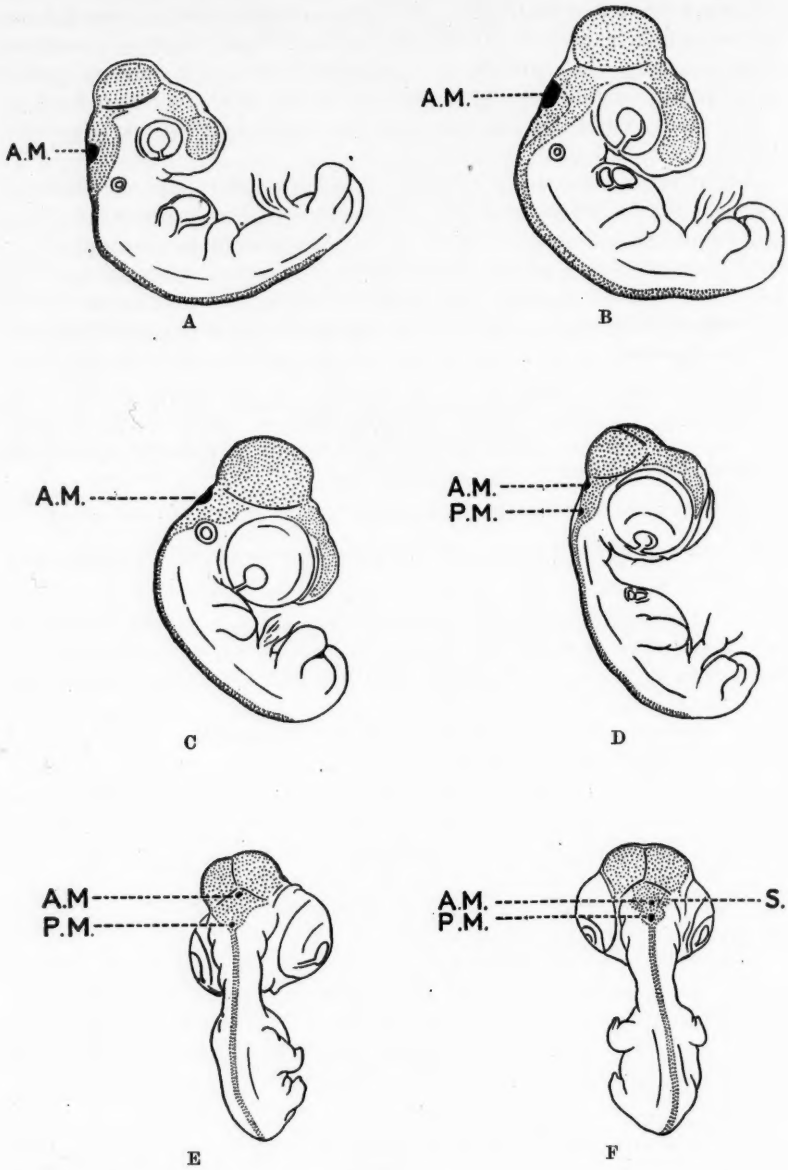
(i) *Injections of a double solution of potassium ferrocyanide and iron ammonium citrate.*

The present section is based on observations, external and histological, of the embryos of series A, B and C. In the embryos of each series injections of a double solution of potassium ferrocyanide and iron ammonium citrate were made into the central nervous system.

In embryos of 3 days' incubation the injected solution was seen to be wholly contained within the central nervous system. It was uniformly distributed within the central canal of the spinal cord and in the ventricular cavities of the brain. The medulla was relatively very large and triangular in shape with its base directed anteriorly and its apex tapering into the spinal cord. The medullary roof was very thin and lay immediately below the integument, thus allowing the dye contained in the fourth ventricle to be more readily seen than elsewhere in the brain stem.

At 3½ days the course of the injected solution was similar to that in a 3 days' embryo, but a median, deeply stained, roughly oval area was now seen in the anterior half of the medullary roof (see Text-fig. 2 A). This area, in virtue of its intense blue colour, stood out in marked contrast to the more diffused colour of the remainder of the medullary roof. No spread of the injected fluid from the central nervous system was evident.

[1] The transparency of the chick embryo in its earlier stages of development enabled the course of the injection fluid to be seen with ease. In older embryos the developing integument obscured the outline of the fluid considerably, and in these cases the material was cleared overnight in cedarwood oil, to render the course of the fluid more easily distinguishable.



Text-fig. 2.

Embryos of $3\frac{3}{4}$ and 4 days' incubation showed a distribution of the injected fluid similar to that in the preceding stage, but the deeply staining oval area in the anterior portion of the medullary roof was relatively larger.

In $4\frac{1}{2}$ days' embryos, again, the injected dye still lay wholly within the central canal of the spinal cord and ventricular system of the brain, but the median, deeply stained area was relatively much larger than previously, occupying the major portion of the anterior part of the medullary roof (see Text-fig. 2B).

At 5 days the distribution of the injected fluid was similar to that in embryos of $4\frac{1}{2}$ days' incubation, but the deeply staining oval area in the medullary roof showed a slight reduction in extent when compared with that of the preceding stages (see Text-fig. 2C).

Embryos of $5\frac{1}{2}$ days showed that the fluid was still confined within the central nervous system, but the median oval area showed even further reduction in extent when compared with that of the preceding stage.

At 6 days the distribution of the injected fluid was similar to that of the previous stage, but the reduction in size of the deeply staining oval area in the medullary roof was even more marked.

In $6\frac{1}{2}$ and $6\frac{3}{4}$ days' embryos the dye was still confined to the spaces of the central nervous system. The median, deeply staining area in the anterior half of the medullary roof was at this stage very small indeed, and a second similar area was now visible in the extreme apical region of the posterior half of the roof (see Text-fig. 2D and E).

Cleared specimens of 7 days' incubation showed that a very slight spread of the dye had occurred from the roof of the fourth ventricle, but the outline of this roof was still clearly visible. The deeply stained area in the anterior part of the roof showed even greater reduction in extent than previously, whilst that in the posterior half was relatively larger (see Text-fig. 2F).

In embryos of $7\frac{1}{2}$ days' incubation the outline of the medullary roof was no longer clearly visible, even in specimens cleared in cedarwood oil. This suggested tentatively that an even more extensive escape of dye had occurred from the fourth ventricle into the overlying spaces. The deeply coloured area in the anterior half of the medullary roof was now so small as to be hardly distinguishable, but that in the posterior half was relatively larger than in the preceding stage.

In cleared specimens of 8, $8\frac{1}{2}$ and 9 days' incubation the outline of the medullary roof was obscured completely; the deeply coloured area in the anterior half of this roof was no longer visible, but that in the posterior half was very large and, in the 9 days' embryos, occupied almost the whole of the apical half of the triangular medullary roof.

The embryos described above were examined histologically with a view to determining the exact nature of the deeply staining oval areas in the roof of the fourth ventricle. At the same time, an attempt was made to discover any possible correlation between the time of appearance of these areas, the spread

of the injected dye from the central nervous system, and the development of the choroid plexuses and meningeal spaces.

In embryos of 3 days' incubation the wall of the brain stem consisted chiefly of ependyma only, or of ependyma associated with a narrow stratum of overlying nerve tissue. The roof of the third and fourth ventricles was in each case continuous and unfolded throughout and showed no indication of a choroid formation. The roof of the third ventricle consisted of an ependymal layer, 3–4 cells deep. The outline of the individual cells was difficult to distinguish owing to the dense granulation of the deeply staining cell cytoplasm. The nuclei were round or slightly elongated in a plane at right angles to the longitudinal axis of the body, and possessed a deeply staining nuclear membrane and 2–3 nucleoli in the chromatin mesh. The roof of the fourth ventricle was triangular in shape, the wide base being directed anteriorly, and the narrow apex posteriorly. The greater part of this roof was extremely thin, consisting of a single layer of low cuboidal cells with granular cytoplasm and round well-defined nuclei touching both the inner and outer cell walls. The nucleoli and nuclear membrane were intensely stained, in contradistinction to the pale nucleoplasm. In the anterior half of this roof a small median oval area could be distinguished, where the cells showed a slight tendency to flattening. This area was not sharply delimited from the surrounding epithelium but gradually merged into it (see Text-fig. 7).

At this stage the general mesenchymal tissue consisted of loosely arranged stellate cells enclosing large, irregular intercellular spaces. The cytoplasm of these cells assumed the form of delicate, pale, granular strands radiating from a centrally disposed nucleus. The nuclei were round or slightly elongated and possessed a deeply staining nuclear membrane, and 2–3 nucleoli in their chromatin mesh. Where the mesenchymal tissue abutted on to the embryonic brain, its cells showed a slight tendency to elongation, the cytoplasmic strands lying parallel to the brain surface.

The precipitated Prussian blue granules were seen wholly within the central nervous system, no extension of the dye into the surrounding mesenchyme being evident. The granules were distributed in a uniform manner, chiefly within the central canal of the spinal cord and in the fourth ventricle of the brain, and showed no condensation associated with any particular area in the brain stem. The distribution of the granules in the cavities of the optic lobes, thalamencephalon, and cerebral hemispheres was very sparse.

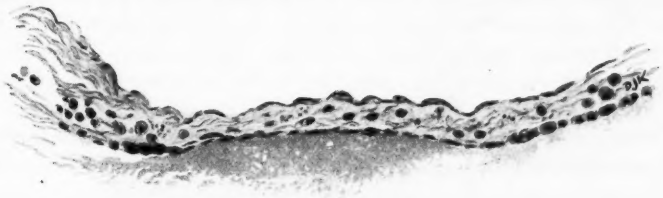
In embryos of $3\frac{1}{2}$ days' incubation the slightly differentiated median area in the roof of the fourth ventricle was now abruptly demarcated from the surrounding roof epithelium. The cells of this area were extremely flat, the granular cytoplasm forming a thin investment around the elongated nucleus. Apart from a marked difference in shape, these cells resembled those of the surrounding epithelium, showing a similar degree of granulation and richness of nuclear chromatin material.

Where the mesenchyme abutted on to the brain surface its cells were not

only elongated as in the previous stage, but also showed a slight degree of condensation, and were more compactly arranged than elsewhere. The narrow ill-defined zone thus formed adhered closely to the brain surface and intimately followed its outline.

The injected fluid was again strictly confined within the central nervous system but no longer exhibited the same uniformity of distribution within the cerebral ventricles as in the preceding stage. There was a dense accumulation of blue granules intermingled with coagulum, adhering to, and co-extensive with, the ventricular surface of the thin membranous area in the roof of the fourth ventricle. The individual cells of this area, however, were entirely free of the dye.

Embryos of $3\frac{3}{4}$ days' incubation presented the same essential features as in the preceding specimen, but the membranous area showed a slight increase in size. It was, however, still sharply delimited from the surrounding epithelium (see Pl. I, fig. 1 and Text-fig. 3).



Text-fig. 3.

The incipient zone of mesenchyme condensation was still narrow but a little more clearly defined from the surrounding mesenchyme than in the previous stage. Further, this zone now showed the first indication of vascularization, minute capillaries being evident in its narrow, intercellular meshes. These capillaries, however, in their distribution bore no relation to the differentiated membranous area in the roof of the fourth ventricle. Where the zone overlay this thin area its cells were less compactly arranged than elsewhere, and showed merely a slight degree of condensation or none at all, so that over this area the zone was hardly distinguishable as a separate entity from the surrounding mesenchyme.

The injected dye still lay wholly within the central nervous system, and showed a particularly dense accumulation below the membranous area in the roof of the fourth ventricle. The cells of this area, however, showed no impregnation by the dye.

In 4 days' embryos the histological appearance of the brain and distribution of mesenchyme were similar to those of a $3\frac{3}{4}$ days' embryo. The injected fluid was still confined to the central canal of the spinal cord and ventricular system of the brain, no invasion of the periaxial mesenchyme being apparent. A heavy condensation of the dye still adhered to the ventricular surface of the membranous area in the roof of the fourth ventricle.

At 4½ days the wall of the brain was thicker than in preceding stages, the greater part consisting throughout of ependyma, associated with a stratum of nerve tissue of variable width. The dorso-mesial walls of the cerebral hemispheres and the roof of the third ventricle, however, were narrower than elsewhere, but still showed no indication of a choroid formation. The myelencephalic roof was still smooth and unfolded. The median membranous area in its anterior portion was slightly larger than in the preceding stages, but was still abruptly demarcated from the surrounding epithelium.

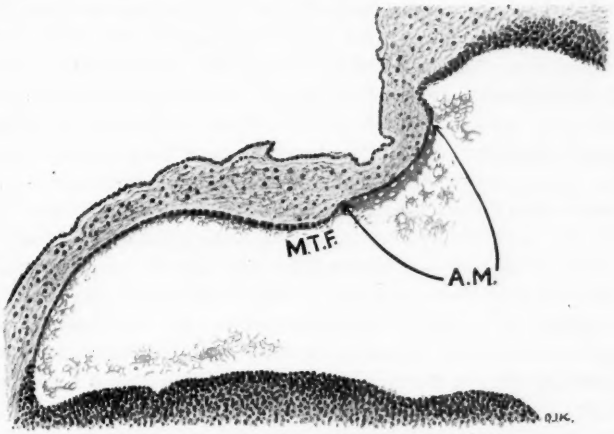
The zone of mesenchyme condensation surrounding the brain was still narrow but more clearly defined than hitherto, and showed a higher degree of vascularization. The general mesenchyme overlying the roof of the fourth ventricle was more loose-meshed than elsewhere and still showed little or no condensation where it actually abutted on to the membranous area in the anterior portion of this roof.

As in earlier embryos, the dye was still confined within the central nervous system, but had now extended anteriorly in uniform distribution to the furthermost recesses of the lateral ventricles. A particularly dense accumulation of blue granules was still apparent below the membranous area in the roof of the fourth ventricle.

In embryos of 5 and 5½ days' incubation the roof of the thalamencephalon was thinner than in the previous stages, consisting only of a narrow ependymal layer 1–2 cells deep, but it was still smooth and unfolded. The epithelial roof of the fourth ventricle was still continuous throughout and showed no indication of a choroid formation. This roof was now clearly divided into anterior and posterior halves by a slight depression in the transverse median plane, and the cells participating in this fold differed from the typical epithelial roof cells in that they tended to be columnar rather than cuboidal. The thin membranous area in the anterior portion of the roof was somewhat smaller than in the preceding stage, but was still sharply delimited from the surrounding cuboidal epithelium. The greater part of the roof posterior to the transverse median fold consisted of cells which showed an unmistakable tendency to flattening, being very low cuboidal in shape, but their nuclei were still round or a little elongated (see Text-fig. 4). This slightly differentiated posterior area differed from the anterior membranous area in that its cells were not so flat and the nuclei still retained, on the whole, their rounded shape; moreover, it was not sharply delimited from the surrounding epithelium but showed gradual mergence with it.

The zone of mesenchyme condensation was still narrow but was now clearly visible as an entity distinct from the general mesenchyme from which it had originated. It consisted of 2–3 layers of cells, more densely compacted together than hitherto. The protoplasmic strands of the cells lay parallel to the brain surface or at right angles to it, and the intercellular meshes so formed contained small capillaries. Over the thin thalamencephalic roof and over the membranous areas in the roof of the fourth ventricle this zone was so narrow and ill-defined as to be hardly distinguishable. A slight indication of a second

outer zone of condensation was apparent in the general mesenchyme in certain localized areas, chiefly in front of the cerebral hemispheres and on the dorsal and ventral surfaces of the thalamencephalon. Where this zone was in process of formation it was very irregular and ill-defined, merging imperceptibly into the general mesenchyme. It was separated from the narrow inner zone by an intermediate region of mesenchymal tissue of variable width. In this newly forming outer zone the typical mesenchyme cells were more compactly arranged than elsewhere and showed a tendency to elongation in a plane parallel to the longitudinal axis of the body. This zone showed no indication of vascularization, its narrow and elongated intercellular meshes being entirely free of blood capillaries. The intermediate zone consisted of typical loose-meshed stellate mesenchyme cells and contained minute capillaries. These



Text-fig. 4.

were uniformly distributed throughout this zone and showed no special concentration over the differentiated areas in the roof of the fourth ventricle.

The injected dye was still confined within the central nervous system, and was particularly condensed below the membranous area in the anterior region of the roof of the fourth ventricle. No such condensation was evident below the slightly flattened area in the posterior region of this roof, but merely a fine distribution of blue granules closely adhering to its ventricular surface.

In embryos of 6 days' incubation the thin roof of the thalamencephalon presented a slightly undulating appearance, forming 2–3 shallow irregular folds, invaded by the overlying mesenchyme. The transverse median fold separating the myelencephalic roof into anterior and posterior regions was more emphasized than previously. Further, the anterior membranous area of this same roof had diminished considerably in extent, but was still sharply

delimited from the surrounding cuboidal epithelium. The posterior slightly flattened area was more extensive than in the preceding stage and, on the whole, exceeded the anterior area both in length and width. It still showed no clear demarcation from the surrounding epithelium. Apart from the transverse median depression, the roof of the fourth ventricle showed no indication of folding and was continuous throughout.

The inner zone of condensation was still narrow, yet clearly defined, except over the thin thalamencephalic roof and over the membranous areas in the roof of the fourth ventricle. The mesenchyme abutting on to these surfaces still showed little or no condensation. The outer zone was more clearly marked than previously but still merged gradually into the surrounding mesenchyme (see Pl. II, fig. 6). This zone had now extended posteriorly over the optic lobes and to the lateral and ventral surfaces of the medulla. In the dorsal medullary region its identity was completely lost in the general mesenchyme. Localized centres of cartilage formation were evident within the substance of this outer zone, particularly in that part of it flanking the medulla. The cells at these points of chondrification tended to a regular disposition, and were isolated from each other by a clear homogeneous matrix. Their nuclei were rounded and well defined, and possessed only a thin investment of pale granular cytoplasm. Where the nervous system approximated closely to the integument, i.e. over the anterior surfaces of the cerebral hemispheres and the dorsal surfaces of the optic lobes, the inner and outer zones lay in such close juxtaposition that the intermediate zone of loose-meshed mesenchyme was almost completely obliterated.

The injected fluid was still strictly confined within the spaces of the central nervous system, and showed the same degree of concentration below the membranous areas of the myelencephalic roof as in the previous stage, but the accumulation of blue granules below the posterior area was slightly denser.

In embryos of 6½ days the incipient folding of the thalamencephalic roof was even more pronounced than previously. The transverse median fold in the roof of the fourth ventricle was deeper than in the preceding stage and its cells were more columnar in shape. The anterior membranous area in this roof showed even further reduction in extent, but was still sharply delimited from the surrounding epithelium. The posterior area, on the other hand, was slightly larger than in the previous stage, but again merged gradually into the general roof epithelium. Its cells were still very low cuboidal in shape with round or only slightly elongated nuclei, but those at the extreme posterior region of this area were extremely flat and resembled the pavement cells of the anterior area.

At this stage the narrow inner zone of mesenchyme condensation showed well-marked intrazonal spaces. Its cells were extremely flat and elongated, and arranged in two ill-defined layers parallel to the brain surface. The very narrow spaces between the cells were traversed by delicate protoplasmic trabeculae arising from the cells of both the inner and outer layers. The numerous intercellular meshes so formed contained small capillaries and consequently this zone, particularly around the cerebral hemispheres, had the appearance of

a chain of minute vascular lumina. Where the mesenchyme cells of this zone abutted on to the thin thalamencephalic roof and membranous areas in the roof of the fourth ventricle they showed little or no condensation, so that the zone was virtually absent in these regions. The outer zone of mesenchyme condensation was more clearly defined than in previous stages and had now extended round the entire brain stem. It was still irregular, however, and was separated from the inner zone by an intermediate layer of typical loose-meshed mesenchyme of variable width. Occasionally the two condensation zones were separated by large irregular spaces containing isolated mesenchyme cells, indicating that these spaces had originated by the breaking down of small portions of the mesenchymal tissue and subsequent confluence of the intercellular spaces.

The injected dye was still retained within the spaces of the central nervous system. A particularly dense accumulation of blue granules and coagulum was still seen adhering to, and coextensive with, the ventricular surface of the anterior membranous area. Further, a heavy condensation was now evident below the extremely flat cells at the apex of the slightly differentiated posterior membranous area.

In embryos of 6¾ days the invaginations of the thalamencephalic roof were more pronounced than previously, and extended forward for a very short distance into the lateral ventricles via the foramina of Munro. The anterior membranous area of the myelencephalic roof was smaller than in the preceding stage, and its border no longer showed an abrupt transition from the surrounding epithelium but gradually merged into it. The posterior membranous area, on the other hand, was now sharply delimited from the general roof epithelium and showed considerable increase in extent. Its cells were extremely flat and possessed narrow elongated nuclei with a thin investment of cytoplasm (see Pl. I, fig. 2).

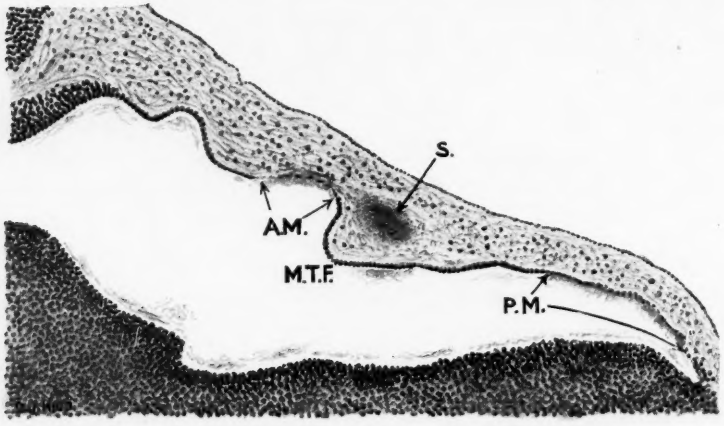
The outer zone of mesenchyme condensation was a little more clearly defined than previously. It showed a slight increase in width and a higher degree of cartilage formation within its substance.

The injected fluid was still confined within the central nervous system, and was particularly condensed below the membranous areas in the roof of the fourth ventricle.

In 7 days' embryos the folds of the thalamencephalic roof were more pronounced than in the preceding stage and, as they extended forward into the lateral ventricles, showed a slight degree of branching, forming incipient choroid villi. In transverse section each villus consisted of an axial core of loose-meshed mesenchyme containing minute capillaries in its intercellular spaces and surrounded by an epithelium 1–2 cells deep. The cells were columnar in shape with granular cytoplasm and round well-defined nuclei. The median transverse fold in the roof of the fourth ventricle was deeper than hitherto and its cells were more markedly columnar. The anterior membranous area was smaller than previously but the posterior area, on the other hand, was larger

and still showed an abrupt transition from the surrounding roof epithelium (see Text-fig. 5).

The distribution of mesenchyme was similar to that in the preceding stage, but the inner zone of condensation was more clearly defined and highly vascular. Where it overlay the thin thalamencephalic roof and the membranous areas in the roof of the fourth ventricle it showed little or no condensation and was, therefore, virtually absent over these regions. The outer zone still merged gradually into the surrounding mesenchyme; it enclosed the brain without closely following its outline and was completely non-vascular. This zone showed a higher degree of cartilage formation within its substance than in the previous stage. The intermediate zone consisted of typical loose-



Text-fig. 5.

meshed mesenchyme cells with minute capillaries scattered in the intercellular meshes. These capillaries, in their distribution, bore no relation to the differentiated roof areas of the fourth ventricle.

Some of the dye, which in all previous stages had been strictly confined to the central canal of the spinal cord and ventricular system of the brain, had at this stage invaded the dorsal medullary mesenchyme (see Text-fig. 5). Apparently the site of fluid escape was the anterior membranous area in the roof of the fourth ventricle. Not only was there a dense accumulation of blue granules below this area, but its cells were actually impregnated with the dye. Further, in the mesenchyme immediately above this thin area there was a particularly dense accumulation of blue granules. Since the inner zone of mesenchyme condensation was virtually absent over the membranous areas in the roof of the fourth ventricle, the dye escaped directly into the meshes of the intermediate zone and did not invade the spaces within the inner zone of condensation. The blood capillaries of the intermediate zone were also entirely

free of the dye, although here and there a slight accumulation of blue granules was evident, adhering to the outer surface of the capillary wall. A heavy precipitation of the injected fluid was also seen below the posterior membranous area. There was, however, no evidence of escape from this region, its cells showing no invasion by the dye. Neither was there evidence of fluid escape from any other point in the ependymal roof of the fourth ventricle. The cuboidal cells of this layer were entirely free of the dye, and exhibited only a fine granular distribution on their ventricular surface.

In embryos of 7½ days' incubation the folding of the thalamencephalic roof was even more marked than in the preceding stage, the folds being slenderer and exhibiting a higher degree of branching. The median transverse invagination in the roof of the fourth ventricle was deeper and the columnar appearance of its constituent cells more pronounced. The ventro-lateral walls of this fold showed a slight degree of undulation, but no true choroid villi were apparent. The anterior membranous area of this roof was even smaller than in the preceding stage, but the posterior area was relatively larger.

The outer zone of mesenchyme condensation was wider and more clearly defined than in the previous stage. The mesenchyme of the intermediate zone immediately above the differentiated roof areas of the fourth ventricle was more loose-meshed than elsewhere and contained large irregular spaces. The capillaries in this zone now tended to be more concentrated in the dorsal medullary region.

The extra-ventricular spread of the injected fluid was slightly more marked than previously. Anteriorly it had extended as far as the cerebellar anlage, and posteriorly as far as the median transverse fold in the medullary roof. The blue granules lay in the meshes of the intermediate zone and did not invade the inner zone of condensation.

In 8 days' embryos, as in preceding stages, the thalamencephalic roof consisted of an ependymal layer only 2–3 cells deep. The degree of folding of this roof, however, was much more marked than hitherto, and the long narrow branching choroid villi so formed extended forward into the lateral ventricles via the foramina of Munro (see Text-fig. 8). The roof of the fourth ventricle showed striking morphological changes. The median transverse fold was extremely well developed and extended almost to the floor of the ventricle. This fold was lined by the inner zone of condensation, and was supported by mesenchymal tissue continuous with that of the intermediate zone. Its walls consisted of columnar cells with granular cytoplasm and round well-defined nuclei. The undulations in the floor of this fold, first recorded in 7½ days' embryos, were here much more pronounced, forming short unbranched incipient villi. These were concentrated in two ill-defined clusters, one at each side of the floor. The anterior portion of the roof of the fourth ventricle was much shorter than in the preceding stage, owing apparently to the encroachment of the developing cerebellar anlage. A median membranous area was still evident in this anterior region but was much smaller in extent than previously. The

posterior membranous area, on the other hand, was relatively much larger and occupied practically the whole of the roof region posterior to the median fold (see Text-fig. 9).

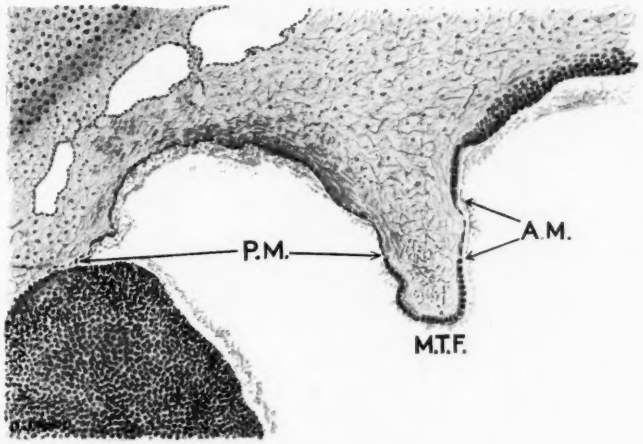
The outer zone of mesenchyme condensation was wider and a little more clearly defined than in the preceding stage. Where the intermediate zone abutted on to the areas of chondrification in the outer zone its cells showed a slight condensation. They were slightly elongated in a plane parallel to the inner surface of the developing bands of cartilage, thus forming an extremely narrow ill-defined zone of fibrous appearance.

The extra-ventricular distribution of the injected dye was more extensive than in the previous stage. It extended for a short distance over the posterior lip of the cerebellar anlage, and even invaded the mesenchyme lying in the upper region of the transverse median fold in the roof of the fourth ventricle. The inner zone of condensation still showed no invasion by the dye. Apparently the posterior membranous area in the medullary roof was now functioning as the site of fluid escape. There was a particularly dense accumulation of blue granules adhering to its ventricular surface. Its cells were deeply impregnated with the dye, and the mesenchyme immediately above this area was more intensely stained than elsewhere. Below the anterior area there was little condensation of blue granules, its cells showed scarcely any impregnation with the dye and the overlying mesenchyme was only slightly coloured.

In embryos of $8\frac{1}{2}$ days' incubation the choroid villi of the lateral ventricles exhibited a higher degree of branching than previously. The incipient villi in the floor of the median fold of the medullary roof were more pronounced than in the preceding specimen, and were slightly branched (see Pl. I, fig. 3). The anterior portion of the medullary roof was even shorter in longitudinal section, bringing the posterior lip of the cerebellum near to the transverse median fold. The membranous area in the anterior region of the roof was very small indeed and was now relegated to the upper part of the anterior wall of the median fold. This membranous area was no longer sharply delimited from the surrounding epithelium but gradually merged into it. Its constituent cells had lost their flat appearance and were now low cuboidal in shape. The posterior membranous area was very extensive, occupying practically the whole of the roof behind the transverse fold. It remained sharply delimited from the surrounding epithelium, and its cells, in contrast to those of the anterior area, were still extremely flat (see Text-fig. 6).

As in previous stages, the inner zone of mesenchyme condensation was still narrow, but nevertheless very clearly defined. Over the membranous areas in the roof of the fourth ventricle it was absent altogether, so that in these regions only a very thin membrane separated the fourth ventricle and its contents from the intercellular meshes of the overlying intermediate zone. The outer zone was wider than previously and showed an even higher degree of cartilage formation. Further, the fibrous layer immediately within this zone was a little more clearly defined.

The extra-ventricular spread of the injected fluid was more pronounced than hitherto. Anteriorly the dye had extended over the cerebellar anlage, and posteriorly for a short distance along the dorsal surface of the spinal cord. The inner zone of condensation still showed no invasion by the dye. Again, the evidence pointed to the posterior membranous area in the roof of the fourth ventricle as being the site of escape. There was a dense accumulation of blue granules, intermingled with coagulum, closely adhering to and coextensive with the ventricular surface of this area. Its cells were deeply impregnated with the dye, and the mesenchyme immediately above showed a very heavy invasion of blue granules. The anterior area, on the other hand, exhibited only a fine granular distribution on its ventricular surface, its cells were entirely free of the dye, and the mesenchyme above this area showed only a slight colouration.

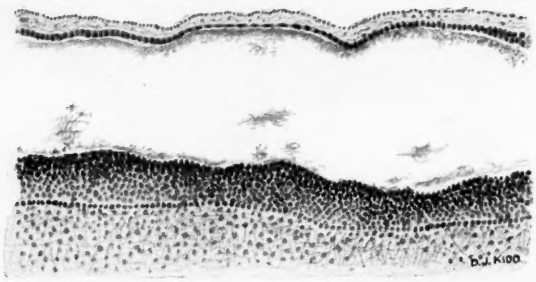


Text-fig. 6.

In embryos of 9 days' incubation the branching of the choroid villi in the lateral and third ventricles was even more emphasized than previously. The developing cerebellum had encroached on almost the whole of the anterior portion of the medullary roof, with the result that the now almost vestigeal membranous area of this region was incorporated yet further into the anterior wall of the transverse median fold. The posterior membranous area, on the other hand, had developed to such an extent that it now occupied almost the whole of the posterior region of the roof, and even extended into the median fold, forming part of its posterior wall (see Pl. I, fig. 4). The walls of the fold below their junction with the membranous roof areas consisted of a single-layered epithelium of cuboidal cells with granular cytoplasm and round well-defined nuclei. This gradually merged into an epithelium 2–3 cells deep, which formed the remainder of the walls and floor of the fold. The cells were columnar

and their cytoplasm was so densely granular that the individual cell boundaries were difficult to distinguish. Their nuclei were round or slightly elongated and possessed deeply staining nucleoli and nuclear membrane. The floor of the fold exhibited a higher degree of villus formation than in the preceding stage, and the branching of the villi was even more pronounced (see Pl. I, fig. 5).

As in previous stages, the brain was surrounded by two zones of mesenchyme condensation separated by an intermediate zone of loose meshed mesenchyme tissue. The inner zone was still narrow and closely adhered to the brain surface. As in all previous stages, the mesenchyme immediately adjacent to the membranous areas in the roof of the fourth ventricle showed little or no condensation, so that above these regions this zone was virtually absent. The width of the intermediate zone varied considerably. On the dorsal surface of the optic lobes and anterior surfaces of the cerebral hemispheres it was very narrow indeed, due to close approximation of the inner and outer zones. Over



Text-fig. 7.

the thalamencephalon, mid-brain and cerebellum it was much wider, but as it extended posteriorly over the medullary roof it then became increasingly narrow. In the mid-dorsal line above the medulla this intermediate zone contained a large irregular longitudinal sinus, extending from the anterior end of the cerebellum to the posterior end of the medulla. The outer zone of condensation was more clearly defined than in the previous stage and showed a higher degree of chondrification. The cells of the intermediate layer immediately adjacent to the cartilaginous bands of the outer zone were even more densely compacted together than previously, forming a definite but narrow zone of fibrous appearance (see Pl. II, fig. 7).

The periaxial spread of the dye was more extensive than hitherto. Anteriorly it had extended over the cerebellum, and posteriorly along the spinal cord for about half of its length. The meshes of the inner zone of condensation were still entirely free of the dye. The injected fluid had obviously escaped through the posterior membranous area in the roof of the fourth ventricle. A heavy condensation of blue granules and coagulum adhered to the ventricular surface of this area, and its cells, together with those of the overlying

mesenchyme, were deeply permeated with the dye. The anterior membranous area showed only a fine granular distribution on its ventricular surface, and its cells were entirely free of the dye. Since the inner zone of condensation was absent over the differentiated roof areas, only a thin membrane separated the fourth ventricle and its contents from the overlying mesenchyme of the intermediate zone; consequently, the escape of dye from the fourth ventricle was directly into the intercellular meshes of this zone. The capillaries and longitudinal sinus of this region showed no invasion by the dye, except at one point where the sinus actually abutted on to the posterior membranous area, and here there was only a very slight, almost negligible, infiltration of the dye through the sinus wall.

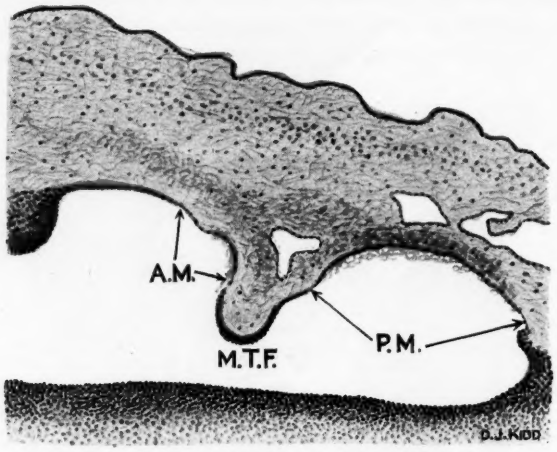
Text-fig. 8.

To summarize: the external appearance of chick embryos in which the central nervous system was injected with the double solution of potassium ferrocyanide and iron ammonium citrate suggested that two specialized areas, one anterior and the other posterior, were present in the roof of the fourth ventricle. These were roughly oval in shape and were distinguishable from the remainder of the roof by their intense colour. The anterior area was first visible in embryos of $3\frac{1}{2}$ days' incubation, and rapidly increased in size until the 5th day. It then commenced a gradual regression, till at 8 days it was no longer visible externally. The first indication of a posterior area was seen in embryos of $6\frac{1}{2}$ days. This gradually increased in size, till at 9 days it occupied the greater part of the posterior region of the roof.

During the earlier stages of development there appeared to be no escape of the injected dye from the central nervous system. At the seventh day of incubation, however, a slight spread was evident from the roof of the fourth ventricle, and this became increasingly marked from the seventh day onward.

These observations of the injected embryos were verified by a detailed histological examination.

The first indication of a differentiated area on the roof of the fourth ventricle was seen in embryos of 3 days' incubation. At this stage it exhibited only a fine distribution of blue granules on its ventricular surface. Therefore no indication of the presence of this area was given by external examination of the embryo. At $3\frac{1}{2}$ days this membranous area was fully differentiated. A heavy



Text-fig. 9.

condensation of blue granules now adhered to its ventricular surface, and therefore its position could be identified externally. Up to the $4\frac{1}{2}$ days' stage this area rapidly increased in size, but at 5 days it showed a slight reduction. It gradually diminished in extent during succeeding stages, and in 9 days' embryos it was practically non-existent. Furthermore, at 5 days the roof of the fourth ventricle was divided by a median transverse fold into anterior and posterior regions. At this stage, too, indications of the differentiation of a second membranous area were evident in the posterior region of the roof. The presence of this second area could not be ascertained externally until the $6\frac{1}{2}$ days' stage, when for the first time a dense accumulation of blue granules was present on its ventricular surface. This posterior membranous area became fully differentiated at $6\frac{3}{4}$ days, and thereafter increased rapidly in extent till at 9 days it occupied almost the whole of the posterior region of the roof. Meanwhile, the median transverse fold in this same roof had become increasingly marked

throughout successive stages, till at 9 days it almost reached the floor of the ventricle. At the same time the cells participating in this fold gradually lost their original cuboidal appearance and became columnar.

In embryos up to and including the age of $6\frac{3}{4}$ days the injected dye remained entirely within the central canal of the spinal cord and ventricular system of the brain. At 7 days, however, there was a slight extension of the dye from the anterior membranous area in the roof of the fourth ventricle into the overlying mesenchyme of the intermediate zone. At $7\frac{1}{2}$ days this spread was slightly more marked, the injected dye still escaping through the anterior membranous area. In embryos of 8 days' incubation the passage of the dye from the fourth ventricle was even more extensive than previously. At this stage, the injected fluid made its escape through the posterior membranous area in the medullary roof, and no longer through the anterior membranous area. At $8\frac{1}{2}$ days there was a very marked periaxial spread of the dye, even along the dorsal surface of the spinal cord, its passage again being through the posterior membranous area. At 9 days the spread of the dye from this same area was even more pronounced. Anteriorly it had invaded the mesenchyme overlying the whole cerebellar anlage, while posteriorly it extended along the cord for a considerable distance.

The first choroid plexuses to develop were those of the third and lateral ventricles. In embryos of 6 days' incubation the thin thalamencephalic roof presented a slightly undulating appearance owing to the formation of 2–3 broad irregular folds. These undulations were more marked at $6\frac{1}{2}$ days, while in embryos of $6\frac{3}{4}$ days they extended anteriorly for a short distance into the lateral ventricles via the foramina of Munro, still in the form of simple unbranched villi. At 7 days, however, the folds exhibited a slight degree of branching, forming an embryonic choroid plexus. From this stage onward the formation of branching choroid villi increased rapidly, till at 9 days they occupied almost the whole of the lateral ventricles. The first indication of the development of the choroid plexus of the fourth ventricle was seen in embryos of $7\frac{1}{2}$ days' incubation. At this stage the ventro-lateral walls of the transverse median fold in the roof of this ventricle showed a slight degree of undulation, but no true villi were apparent. At 8 days the undulations were much more marked, resulting in the formation of short unbranched villi concentrated in two ill-defined clusters in the floor of the fold. In $8\frac{1}{2}$ days' embryos the formation of these villi was more pronounced, and they exhibited a slight tendency to branching, which became even more marked at 9 days.

In 9 days' embryos the brain was enclosed by the developing cranium and two meninges, an inner and an outer, which were separated by an intermediate layer of undifferentiated mesenchyme. The inner meninx was narrow, closely adhered to the surface of the brain, was loosely trabeculated and contained minute capillaries in its intercellular meshes. Where this layer abutted on to the membranous areas in the roof of the fourth ventricle it was so thin as to be almost absent. The outer meninx, on the other hand, lay close to the inner

surface of the embryonic skull; it was wider than the inner membrane, was non-vascular and presented a fibrous appearance. The intermediate zone separating the inner and outer meninges was of variable width and possessed a fairly rich blood supply. Where this zone overlay the roof of the fourth ventricle it contained large irregular spaces.

Both meninges and the cranial anlage originated *in situ* by a process of cellular condensation in the general mesenchyme surrounding the brain. The inner meninx arose as a single membrane from a zone of condensation immediately adjacent to the brain surface. It was first distinguishable in embryos of $3\frac{1}{2}$ days' incubation, but at this stage was narrow and ill-defined. At $4\frac{1}{2}$ days it was still narrow but more clearly visible as a distinct layer, and now showed a slight degree of vascularization. During successive stages of development this zone became increasingly vascular and more clearly defined. The cranial anlage was first visible in embryos of 5 days' incubation where it was represented by an extremely irregular and ill-defined zone of mesenchyme condensation situated at some distance from the inner meninx. At 6 days this zone was more clearly marked, and localized centres of cartilage formation were evident within its substance. In later stages this zone became increasingly well defined and showed a progressive degree of chondrification. Meanwhile, at 8 days the cells of the intermediate zone immediately adjacent to the developing cranium showed a slight degree of condensation, forming a very narrow ill-defined layer of fibrous appearance. At $8\frac{1}{2}$ days this zone was more marked, and at 9 days it was clearly visible as a definite but narrow fibrous membrane immediately within the embryonic cranium.

Owing to the absence of the inner meninx over the membranous areas in the roof of the fourth ventricle, the injected dye, in its passage from this ventricle, escaped directly into the meshes of the intermediate zone. Never at any stage was it seen to invade either the inner or the outer meninx.

(ii) *Injections of a solution of iron ammonium citrate alone*

The present section is based on external and histological observations of the embryos of series D. These were injected with a solution of iron ammonium citrate into the central nervous system. Since a detailed account of the development of the roof of the fourth ventricle, choroid plexuses and meninges has been given in the previous section, a description of the distribution of the injected fluid alone is given below.

In embryos up to and including the age of $6\frac{1}{2}$ days the injected solution was seen to be contained within the central nervous system. In cleared specimens of 7 days' incubation a slight escape of fluid was evident from the roof of the fourth ventricle. At $7\frac{1}{2}$ days this spread was more marked and partly obscured the outline of the medullary roof. In embryos of 8, $8\frac{1}{2}$ and 9 days' incubation the outline of this roof was no longer visible externally, suggesting that an even greater extra-ventricular spread of the injected fluid had occurred.

These observations on the external appearance of the injected embryos were confirmed by histological examination.

In embryos of this series the development of the membranous areas in the roof of the fourth ventricle was similar to that described in embryos of series A, B and C. When these areas were present a heavy condensation of dye was seen adhering to their ventricular surface (see Pl. II, fig. 8).

Until the age of 7 days the injected solution was confined within the central nervous system. At 7 days there was a slight escape of the dye through the anterior membranous area into the overlying mesenchyme between the two zones of condensation (see Pl. II, fig. 9). At 7½ days this spread had increased, but was still through the anterior area. In 8 days' embryos the extension of the dye from the fourth ventricle was even more pronounced than previously. At this stage the posterior area alone functioned as the site of fluid escape. At 8½ days the injected fluid had spread to an even greater extent, its passage still being through the posterior membranous area. In 9 days' embryos the escape of the dye through this same area was even more marked. Anteriorly it had invaded the mesenchyme overlying the optic lobes, while posteriorly it extended along the spinal cord for about half of its length.

The meninges of the brain in embryos of series D showed no invasion by the dye. The injected fluid in its passage from the fourth ventricle escaped directly into the meshes of the intermediate zone.

Thus, embryos in which the central nervous system is injected with a solution of iron ammonium citrate alone yield the same results as those injected with the double solution of potassium ferrocyanide and iron ammonium citrate.

(*b*) *Results of injections of true solutions into the vascular system*

The observations recorded in sections V*a* (i) and V*a* (ii) showed that the first escape of injected fluids from the central nervous system into the surrounding tissue occurred in embryos of 7 days' incubation. At this stage the passage of the dye was through the anterior membranous area in the roof of the fourth ventricle, and from 8 days onwards through the posterior area.

Injections were made into the vascular system in order to ascertain whether the anterior and posterior areas at 7 and 8 days respectively allowed the passage of dye *into* the fourth ventricle from the surrounding tissue. For this purpose two series of embryos were injected, series E and F. In both cases the age limits of the series were 5 and 9 days, thus allowing an ample margin of time before and after the critical ages.

In the embryos of series E, injections of the double solution of potassium ferrocyanide and iron ammonium citrate were made into the left ventricle of the heart. Embryos of series F were similarly injected, but with a solution of iron ammonium citrate alone.

In embryos of both series Prussian blue granules were seen in the capillaries of the head region and even in those of the inner meninx. There was, however,

no evidence of any passage of the dye through the capillary walls into the surrounding tissue. Therefore, it cannot be said whether the membranous areas allow the passage of dye into the fourth ventricle or not. But one fact emerged clearly—there was no passage of fluid from the vascular system into the ventricles of the brain.

VI. GENERAL SUMMARY AND DISCUSSION

The present investigation shows that at certain stages in the development of chick embryos two differentiated areas, one anterior and the other posterior, are present in the roof of the fourth ventricle. The two areas are sharply delimited from the general roof epithelium, and consist of very flat cells with narrow elongated nuclei. These areas can be identified with the area membranacea superior and the area membranacea inferior described by Weed (1917), in the roof of the fourth ventricle of pig and human embryos.

In the chick the anterior area is first differentiated in embryos of $3\frac{1}{2}$ days. It then increases rapidly, till at $4\frac{1}{2}$ days it occupies the greater part of the anterior region of the medullary roof. At 5 days this area shows a slight reduction in extent, and gradually diminishes during successive stages, till at 9 days it is almost non-existent. As in pig and in human embryos, the disappearance of this area is brought about by the encroachment of the developing cerebellum on the anterior portion of the medullary roof. The posterior area first appears in embryos of 5 days' incubation. At $6\frac{3}{4}$ days it is fully differentiated, and thereafter increases rapidly, till at 9 days it occupies the greater part of the medullary roof behind the choroid plexus.

Thus the development of these membranous areas in the roof of the fourth ventricle of the chick shows the same sequence of events as that described by Weed (1917) in pig and in human embryos.

The passage of injected fluids from the central nervous system into the surrounding tissue is first seen in chick embryos of 7 days. At this stage the spread of the fluid is very slight and occurs through the anterior membranous area alone. At $7\frac{1}{2}$ days the escape of the solution is more marked and still takes place through the anterior area. This extra-ventricular spread is much more extensive at 8 days, but the dye now passes through the posterior area only. In embryos of $8\frac{1}{2}$ and 9 days the fluid escapes even more extensively through this same area, spreading over the cerebellum, optic lobes and anterior part of the spinal cord.

From the work of Weed (1917), it appears that the membranous areas in the roof of the fourth ventricle function almost as soon as they are formed. In the chick, however, these areas are fully differentiated several days before they allow of any fluid escape.

According to Keegan (1917), in chick embryos injected with the double solution of potassium ferrocyanide and iron ammonium citrate, no escape of fluid occurs from the central nervous system, even when the choroid plexuses are well developed. We found, however, that in embryos injected with the

double solution there is a definite spread of fluid from the central nervous system, correlated with the development of the choroid plexuses. The first choroid plexuses to develop are those of the third and lateral ventricles. These are fully formed at 7 days, and at this stage the first extra-ventricular spread of the injection fluid occurs. The choroid plexuses of the fourth ventricle are only fully developed between $8\frac{1}{2}$ and 9 days, and this was associated with a further marked escape of fluid from the fourth ventricle.

In the chick, therefore, the first spread of fluid is coincident with the development of the choroid plexuses of the third and lateral ventricles. A similar correlation between the development of the choroid plexuses and escape of injection solution is recorded by Weed (1917) in pig embryos. Here the first spread corresponds with the formation of the choroid plexus of the fourth ventricle, this plexus being the first to develop.

Observations on chick embryos in which the central nervous system was injected with a solution of iron ammonium citrate alone do not agree with the findings of Keegan (1917), who made the same investigation. He states that the citrate solution invariably leaves the fourth ventricle even when no choroid plexuses are present. The present investigation shows a definite correlation between the escape of the dye and the formation of the choroid plexuses. An extra-ventricular spread was never observed before the appearance of a choroid plexus.

The observations herein recorded show that only two meninges are present in the chick, an inner and an outer, separated by a region of undifferentiated mesenchyme. The outer meninx, lying immediately within the embryonic skull, is narrow, non-vascular and presents a fibrous appearance. The inner meninx, closely adhering to the surface of the brain, is narrow, richly vascular and presents a loosely trabeculated appearance, but cannot be divided into two separate membranes.

The outer meninx is obviously the dura mater. From its appearance the inner meninx can be identified with the secondary meninx described in birds by Sterzi (1902), Ariëns-Kappers (1929), and Ariëns-Kappers *et al.* (1936). The development of this inner meninx also shows that it corresponds to the pia-arachnoid of the chick, described by Farrar in 1906.

Never at any stage of development was the inner meninx invaded by solutions injected into the central nervous system. These fluids in their passage from the fourth ventricle escape directly into the meshes of the intermediate zone. Therefore, the present investigation clearly shows that, contrary to the statement of Hansen-Pruss (1923), the inner meninx functions as a single membrane and its spaces are not comparable physiologically to the sub-arachnoid spaces of the Mammalia.

VII. CONCLUSIONS

1. In chick embryos the cerebrospinal fluid, as indicated by the distribution of injected solutions, first escapes from the central nervous system at the seventh day of incubation. In 7 and 7½ days' embryos the passage of the fluid is through a membranous area in the anterior portion of the medullary roof. At 8, 8½ and 9 days the fluid escapes through a similar area in the posterior region of the roof.

2. The first choroid plexuses to develop are those of the third and lateral ventricles. These are fully formed at 7 days, and at this stage the first extraventricular spread of fluid occurs. The choroid plexus of the fourth ventricle is fully developed between 8½ and 9 days, and this is correlated with a further marked escape of fluid from the fourth ventricle. Therefore, the spread of cerebrospinal fluid from the central nervous system is coincident with the development of the choroid plexuses of the brain.

3. Two meninges are present in the chick, an outer dura mater and an inner secondary meninx. The inner meninx, though loosely trabeculated, develops and functions as a single membrane. Its spaces contain no cerebrospinal fluid, and are therefore not comparable with the subarachnoid spaces of the Mammalia.

We wish to thank Mr K. M. Wilson for constructing the apparatus shown in Text-fig. 1, and Mr F. Beckwith for taking the photographs.

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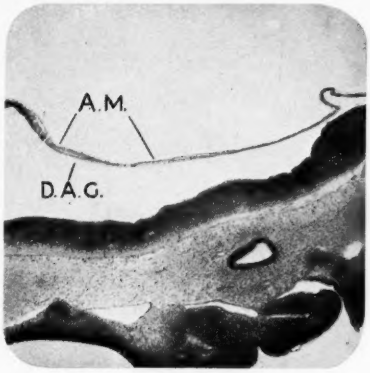

Fig. 1

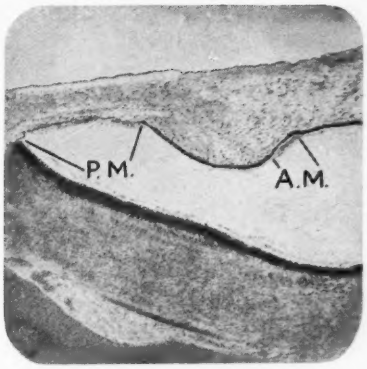

Fig. 2

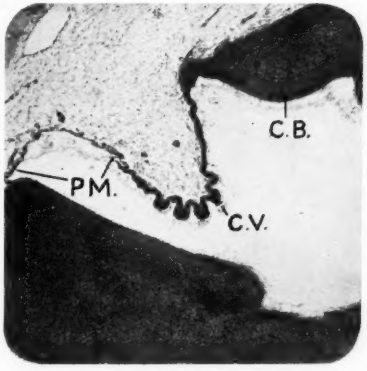

Fig. 3

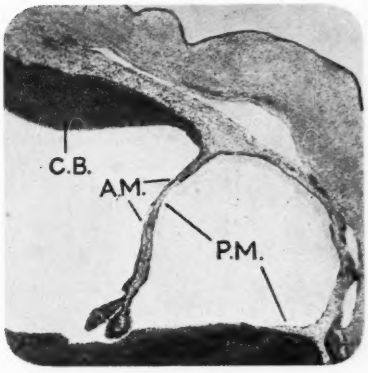

Fig. 4

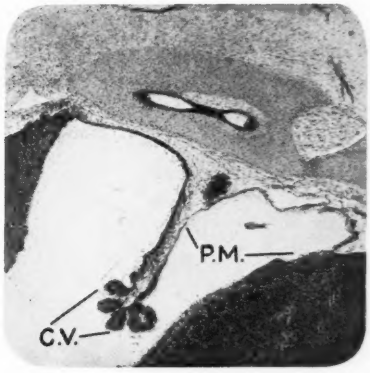

Fig. 5

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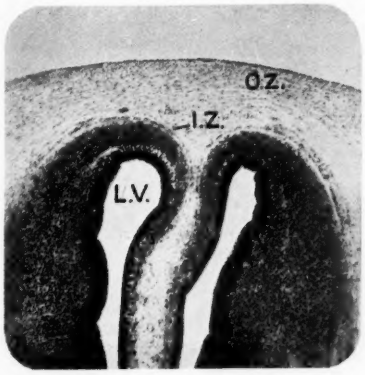

Fig. 6

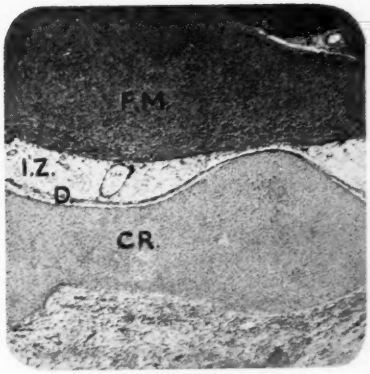

Fig. 7

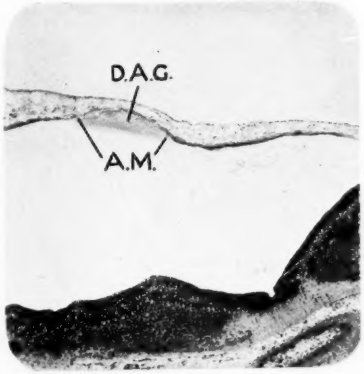

Fig. 8

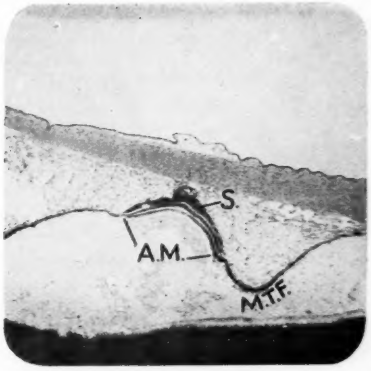

Fig. 9

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EXPLANATION OF PLATES I AND II

Plate I

Fig. 1. Photomicrograph of a longitudinal section of the roof of the fourth ventricle in a 3¾ days' embryo. In this specimen an injection of a double solution of potassium ferrocyanide and iron ammonium citrate was made into the central nervous system. (Acid formaldehyde : Ehrlich's haematoxylin and eosin.) ×31 approx.

Fig. 2. Photomicrograph of a longitudinal section of the roof of the fourth ventricle in a 6¾ days' embryo, injected as in specimen illustrated in fig. 1. (Acid formaldehyde : Ehrlich's haematoxylin and eosin.) ×24 approx.

Fig. 3. Photomicrograph of a longitudinal section of the roof of the fourth ventricle, showing the development of the choroid villi in an 8½ days' embryo, injected as in specimen illustrated in fig. 1. (Acid formaldehyde : Ehrlich's haematoxylin and eosin.) ×38 approx.

Fig. 4. Photomicrograph of a longitudinal section of the roof of the fourth ventricle in a 9 days' embryo, injected as in specimen illustrated in fig. 1. (Acid formaldehyde : Ehrlich's haematoxylin and eosin.) ×28 approx.

Fig. 5. Photomicrograph of a longitudinal section of the roof of the fourth ventricle showing the choroid villi in a 9 days' embryo, injected as in specimen illustrated in fig. 1. (Acid formaldehyde : Ehrlich's haematoxylin and eosin.) ×36 approx.

Plate II

Fig. 6. Photomicrograph of a transverse section of the cerebral hemispheres, showing the inner and outer zones of mesenchyme condensation in a 6 days' embryo. (Bouin's Fluid : Ehrlich's haematoxylin and eosin.) ×37 approx.

Fig. 7. Photomicrograph of part of a longitudinal section of the floor of the medulla, showing the dura mater in a 9 days' embryo. (Acid formaldehyde : Ehrlich's haematoxylin and eosin.) ×37 approx.

Fig. 8. Photomicrograph of a longitudinal section of the roof of the fourth ventricle in a 4¾ days' embryo. In this specimen the central nervous system was injected with a solution of iron ammonium citrate. (Acid formaldehyde and potassium ferrocyanide solution : Ehrlich's haematoxylin and eosin.) ×30 approx.

Fig. 9. Photomicrograph of a longitudinal section of the roof of the fourth ventricle in a 7 day's embryo, injected as in specimen illustrated in fig. 8. (Acid formaldehyde and potassium ferrocyanide solution : Ehrlich's haematoxylin and eosin.) ×24 approx.

ABBREVIATIONS

A.M. Anterior membranous area.
C.B. Cerebellum.
CR. Cranium.
C.V. Choroid villi.
D. Dura mater.
D.A.G. Dense accumulation of blue granules.
F.M. Floor of medulla.
I.Z. Inner zone of mesenchyme condensation.
L.V. Lateral ventricles.
M.T.F. Median transverse fold.
O.Z. Outer zone of mesenchyme condensation.
P.M. Posterior membranous area.
S. Extra-ventricular spread of injected fluid.

THE NERVE SUPPLY OF BONE

By DANIEL J. HURRELL

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Bone has many qualities which suggest that it has a nerve supply. It is one of the most actively living tissues of the body even in the adult. Excepting white fibrous tissue it is the only one which is repaired with its own material. The repair is a highly intelligent and co-ordinated one, a reconstruction. Trabeculae originally laid down to take the stresses and strains to the best mechanical advantage with the minimum of material are taken away and new ones put up in a better place, suggesting that the laying down of bone is a response to stimuli arising through bending or stretching. In old age, with its lessened bodily activity, the bones rarefy. They do so even in youth if the subject is confined to bed, but here they recover again when they begin to be used actively. Too rigid fixation of fracture fragments almost inhibits union. Constant stimuli arising through the use of the bones therefore seem necessary to their maintenance.

The sensitivity of bone is mostly stated to be low, yet fractures and inflammation of bone are very painful. Clinical experience on this subject is summarized by Leriche (1930), who points out that by Böhler's (1929) technique of a single large injection of novocaine between the ends of a fractured bone anaesthesia and relaxation of muscle spasm is quickly produced, even while everything from periosteum to skin is still sensitive, showing that the pain is arising deep in the bone. He also says that osteotomy under local anaesthesia requires the introduction of more novocaine for each few millimetres of cutting, and that the marrow spaces and periosteum are well known to be very sensitive.

The question of the existence of specifically trophic nerves is raised by the occurrence of atrophic processes in bone in such diseases as tabes dorsalis and syringomyelia. The clinicians incline to believe that such nerves do exist. Most of the literature on this aspect of the subject may be found reviewed by de Castro (1925, 1930), Kuré (1936) and Müller (1931), but it may be briefly stated here that the confusion arises from so little having been done to trace the nerves of the "passive tissues" to their origins and terminations. At present no one can say whether an atrophy is due to loss of specifically trophic fibres, or is merely secondary to vasomotor upset, or due to loss of ordinary sensation and the protection which pain affords, or is an atrophy from disuse.

PREVIOUS WORK ON THE NERVES OF BONE

The chief worker on this subject was de Castro, who evolved a technique for silver impregnation of nerves in tissue which had been through decalcifying acids, and described nerves supplying growing bone. He found nerve fibres entering with the irrupting vessels and traced them along to the final capillary loops. As the vessels burrowed deeper he found that they left nerve twigs connected to the osteoblasts by a loop form of ending in contact with the cell protoplasm. All the osteoblasts were not found to have a nerve supply, and this he explains by saying that these cells are probably not discrete units, but are interconnected. He states, however, that "There are no nerves in fully formed bone, because, as the bone consolidates and the osteoblasts become included in it so as to become osteocytes, they lose the nerve supply which they had by a retrograde degeneration of the fibres." By silvering whole rat and mouse heads he traced the nerves of the developing basi-occipital and petrous temporal bones to the superior cervical sympathetic ganglion and the vagus and glossopharyngeal nerves. In his view the nerves of developing bone are from the autonomic nervous system only.

Other workers have confirmed de Castro's findings in growing bone although they were unable to find the special form of ending he described. Schartau (1936) used sections of 40–50μ which showed the general arrangement but no detail. Miskolczy (1926), working mainly on the periosteum, saw nerve fibres entering the mouths of the Haversian canals as well as the bone surface at other points, but he could follow them no farther.

The general textbooks of histology state that there are nerves in the Haversian canals and that they are probably mostly vasomotor, but that some go on to the endosteum and the marrow. Stöhr (1928) makes a similar statement and quotes Miskolczy. He also mentions Lushka's finding that the nerves to the vertebral bodies come from the cranial and sympathetic nerves.

TECHNIQUE AND MATERIAL

The working out of a method of staining nerves in bone was the first problem to be solved. No claim is made that this has been fully done, but that the way has been cleared for further work.

Only block impregnation with silver nitrate and serial sectioning in paraffin were tried extensively, since it was realized that only serial sections could be the basis of nerve demonstration in bone. The Bielschowsky-Gros and Cajal methods for frozen sections were tried after fixation-decalcification by de Castro's method, but without success.

The ordinary methods for silver impregnation very seldom show any nerve fibres even in muscle, etc., after the block has been through decalcifying acid. Stewart (1936) reported one such success using Cajal's ammoniated alcohol technique on decalcified dentine. Cajal (1933) says that nitric acid in formol, used for 1 day on embryos and small foetuses, seems actually to better

the neurofibrillar action of silver. Huber & Guild (1913) thought the failure of silver methods on bone was due to imperfect fixation in the interior of solid bone. They used a slightly modified Ranson technique and injected the fixative into the arteries of anaesthetized animals. They were able to stain nerves passing through bones but have described none distributed to bone or its contents. Their method is unfavourably criticized by de Castro and was not tried by the writer.

In the writer's hands the only methods which can be relied upon to show nerve fibres anywhere with constancy after decalcification were de Castro's or those using his addition of hypnotic drugs, chloral hydrate, somnifaine, etc., to the fixing-decalcifying bath. But de Castro's method, even when it gave a strong impregnation of the nerves of the soft parts of a block, never showed nerve fibres in bone matrix. As implied above, he himself never succeeded in impregnating such nerves.

It became clear by degrees that there were probably two causes at work preventing the staining of nerve fibres after acids, the first being some effect overcome by de Castro's addition of the hypnotics to the acid bath, allowing the nerves of the soft parts to be shown, the second being simple failure of the silver nitrate and other solutions to penetrate the piece of bone after its fibrous matrix had been swollen during decalcification, washing, etc. The use of alum to prevent the swelling here suggested itself.

It also seemed that, since de Castro's method often failed to show up very fine fibres and nerve endings, more strongly acting methods such as those in use for embryonic material should be tried after fixing and decalcifying by de Castro's method. The combination thus arrived at is given in the table below, and a summary of materials and other technique tried is given later. The method given is de Castro's fixation-decalcification, using a high alcohol because de Castro's 60 per cent alcohol allows maceration if used for more than a few days, followed by Cajal's ammoniated silver technique with a stage of 5 per cent potash alum. This method gave one strong impregnation of nerves in bone matrix, but all the soft parts of the block are choked with a heavy precipitate of silver. Attempts to repeat the impregnation have all failed, and there is always much precipitation of the silver, so that it is certain that, though one success has shown that the reason for using alum to prevent swelling of the bone matrix by the acid is probably correct, alum is not a good agent for the purpose because it interferes with the working of the silver afterwards.

TECHNIQUE USED

1.	Fix in 100 c.c. 96 per cent alcohol + 4 c.c. somnifaine + 6 c.c. nitric acid, until decalcified	
2.	100 c.c. 96 per cent alcohol + 2 drops liquor ammoniae fort.	24 hours
3.	50 per cent formol	24 hours
4.	100 c.c. 96 per cent alcohol + 2 drops liquor ammoniae fort.	24 hours
5.	5 per cent potash alum	24 hours
6.	Pyridin	3 days

7. Distilled water, many changes . . . . . 2 days
8. 3 per cent. silver nitrate at 37° C. in darkness . . . . . 7 days
9. Ammoniated silver nitrate, changed daily . . . . . 2 days
   (10 c.c. of 10 per cent silver nitrate,
   20 drops of 40 per cent NaOH; wash precipitate in three changes of distilled water,
   400 c.c. distilled water,
   ammonia till precipitate is almost all dissolved.)
10. Wash in distilled water + 0·2 per cent glacial acetic acid . . . . . 12–24 hours
11. 20 per cent formol . . . . . 3 days
    Wash and embed in paraffin.

SUMMARY OF MATERIALS AND TECHNIQUES EXPERIMENTED WITH, AND THEIR RESULTS

At first the humerus and femur of new-born kittens were used on account of their small size and ease of decalcification, and about twenty blocks put through most of the established techniques, Cajal, de Castro, Ranson, Blair & Davies (1935) modification, Bielschowsky, etc., trying the effects of different acids, longer or shorter times in pyridin, silver nitrate, etc.

As it became clear that there was no great advantage in using young bone and that adult bone might differ from young bone, use was next made of pieces of the chest wall of adult guinea-pigs, rats, mice and cats. Small blocks, each a part of two ribs and the space between, were put down in batches of one to two dozen in the techniques and variations in the last paragraph, as well as in others from the large numbers now published, such as Agduhr's modification of Bielschowsky's technique, Cajal's ammoniated silver technique, de Castro's with 1–2 days pyridin after the ammoniated alcohol (a useful introduction in the much better differentiation it gives between nerve and connective tissue), and de Castro's with a stage of 50 per cent glycerin in distilled water for 7 days after the ammoniated alcohol, which gives an even better differentiation than pyridin, leaving bone matrix a clear yellow. None of these gave good impregnations of nerves anywhere except de Castro's. Even it falls short on fine twigs and terminations and fails to show nerves in bone matrix.

Because these small ribs show almost no Haversian bone adult cat femur was next tried. This bone has about twice as much ground lamellae as human, but the Haversian systems are well developed. It was at this time that the use of alum suggested itself, so six pieces were used in modifications of Cajal's ammoniated silver method, with and without alum. Block 1 was fixed in ammoniated alcohol, decalcified in 6 per cent nitric acid in the formol, and then carried on as in the table above. Block 2 was fixed in alcohol with 2 c.c. per cent of somnifaine, decalcified in the formol, and the rest as in the table. Block 3 was treated as in the table. Blocks 4, 5 and 6 were given the same treatment as 1, 2 and 3 but without alum. Block 3, a piece of the femur of a fully grown cat, age unknown, but without signs of growth in the bone, is the one specimen obtained showing nerve fibres in the bone matrix.

The method is long, since decalcification in high-percentage alcohol is slow. It has been repeated six times unmodified and each time with 5–6 blocks in modifications such as putting the alum bath between different stages, and using smaller bones such as rat and guinea-pig, but without further success. The successful specimen was obtained from a cat. The tissues of this animal were anoxaemic and contained chloralose, death having been brought about by this anaesthetic. The technique has been tried again on similar pieces of bone and on bone from a rat killed with chloralose, but without success.

It seems very probable, however, from this one result and from experience with de Castro's technique which stains nerves so constantly in the soft tissues of a block, that the problem remaining to be solved is a purely physical one, to prevent swelling of the fibres of the bone matrix and at the same time to avoid shrinking it as a whole.

FINDINGS

It was felt very undesirable to base much on one specimen. Publication was, therefore, delayed for more than a year while efforts were being made to produce more results. The block was cut longitudinally and serially at 12μ, and the sections were thoroughly examined and shown to others. There is no room for doubt that the structures described are nerves. The nerve impregnation and the differentiation from connective tissue are good even where Sharpey's fibres are shown. These are brush-like and more or less at right angles to the periosteum, while the nerves run in or parallel to the Haversian canals, finally diverging from them into the matrix. One can follow the nerve fibres from the periosteum along the canals to their endings in the matrix and close to the bone cells. The nerves which enter with the nutrient vessels by the main nutrient foramen seem all destined for the marrow or its vessels. The fibres destined for the bone matrix accompany the vessels which enter the Haversian canals here and there over the surface. At all these points one or two fibres enter closely applied to the vessels while others run into the bone parallel to the canals, but often as much as the width of the canal away from it, suggesting that the canal had once been wider and that the nerve fibres had become included in the bone (Pl. II, figs. 2, 3, 4). In the canals in the depths of the bone the nerves retain this relationship, some lying inside and some just outside the lumen (Pl. II, fig. 6, and Pl. I, fig. 4). The actual boundary of a canal is hard to define, and the presence of lacunae has been taken to mark definite bone from canal or from periosteum. There is usually also an indication of surface in a darker deposit of silver.

The nerve fibres are then distributed to either side, covering well over half the distance to the next adjacent canal (Pl. II, fig. 1, and Pl. I, figs. 3, 6). There is even an indication in places of communication between the nerves of such a pair of canals. The branching is free and irregular. In many places there are curious coiled hanks of fibres not far from the canals, with thick fibres giving off thin terminal twigs (Pl. I, fig. 5). The fibres lying in the bone

matrix show most of the histological characters of unmyelinated nerves, varicosities, alternate thickening and thinning, beading due to neurolemmar nuclei, etc. (Pl. I, figs. 1, 2, and Pl. II, fig. 1). They are of medium thickness. The thickness of nerve fibres in the same region and same species of animal seems, however, to vary greatly with the technique used. They appear thicker after nitric than after formic acid.

The endings of the fibres in the matrix show the same characters as those always found by the writer in periosteum using de Castro's method. Most of the fibres grow thinner and thinner and end in scattered specks of silver which can be traced no farther with an oil immersion lens and 20× eyepieces in a binocular microscope. Other fibres run into very close relationship with the bone cells (Pl. I, figs. 1, 2, and Pl. II, fig. 1), but with the same power nothing could ever be seen resembling the ring terminals described by de Castro on the osteoblasts of growing bone.

An idea of the richness of the supply may be gained from the fact that in the best part of the block no part of the field of a 1/6 in. lens is without nerve fibres. Here also the nerves extend to about two-thirds of the thickness of the wall of the shaft, but nothing should be based on this, for the impregnation is obviously incomplete in other places.

With only one impregnation any attempt at interpreting what function these fibres can have must only be very tentative. De Castro's work has established that nerve fibres are at least closely connected with the osteoblasts of growing bone. He suggested that these nerves are probably concerned with the modelling of the bone and may even be the site of action of the endocrine secretions affecting bone. Without being too speculative one may say that the endings in adult bone described in this paper suggest, in conjunction with the properties of bone mentioned in the introduction, that they may be the sensory and effector endings required for a reflex control of bone growth and maintenance. The free nerve endings are a type of termination associated with protopathic sensation (the only type of sensation elicited in bone clinically), and those in relation with the osteocytes are evidently the same as those seen by de Castro related to the osteoblasts of young bone, but supposed by him to degenerate when these cells became enclosed in the bone as osteocytes.

The nerve supply of bone marrow has been partly worked out by de Castro and others. The writer can confirm de Castro's findings that most of its nerves go to the blood vessels, but that there are others which end among the marrow cells, their manner of ending being unknown. Further work will be carried out on them at the same time as that obviously still much needed on the nerves of bone matrix.

SUMMARY

1. Nerve fibres were traced into and along the Haversian canals of adult bone and into the bone matrix. Their distribution, characters and endings in the bone matrix are described. Some fibres end blindly in the bone matrix, some in close relation with the bone cells.

2. A short account of the materials and techniques experimented with is given, leading to those eventually successful in one case, a piece of adult cat femur and a combination of de Castro's method with Cajal's ammoniated silver method, with a stage of 5 per cent potash alum.

3. De Castro's addition of hypnotics to the decalcifying bath solves the difficulty of producing silver impregnation of nerves after decalcification. The remaining difficulty is the finding of the most suitable agent which will prevent the swelling of the white fibrous matrix of the bone in the acid, so that it may be permeable to the reagents used later. Alum is the type of substance needed for the purpose but it interferes with the working of the silver afterwards.

4. A suggestion is put forward tentatively that the nerve fibres found may be the two ends of a reflex arc governing bone growth and maintenance.

My grateful thanks are due to Profs. Blair and Davies at whose suggestion the work was commenced, as well as to Prof. Nicol and Mr Stibbe for their advice and criticism.

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EXPLANATION OF PLATES I AND II

All sections are longitudinal

Plate I

Fig. 1. Terminal piece of a nerve in bone matrix, showing the histological characters. On the left of the field a fibre is seen to pass practically in contact with a bone cell (a very common feature in these sections) and then to pass on to another bone cell. × 1095.

Fig. 2. End of a nerve fibre in the bone matrix. Shows the histological characters, varicosity and beading. A little below the middle of the left side of the field is the outline of a bone lacuna to which the nerve fibre ends are going. × 1500.

Fig. 3. Nerve fibre leaving a canal and running into the matrix. × 840.

Fig. 4. Section tangential to a Haversian canal. Shows nerves accompanying the canal but lying in the bone outside it. × 840.

Fig. 5. Coiled hank of nerve with fine bare endings. The section is just tangential to a canal (dark upper part of background). × 840.

Fig. 6. Shows the extent of the distribution of a nerve fibre in the matrix between the two Haversian canals marked by arrows. × 588.

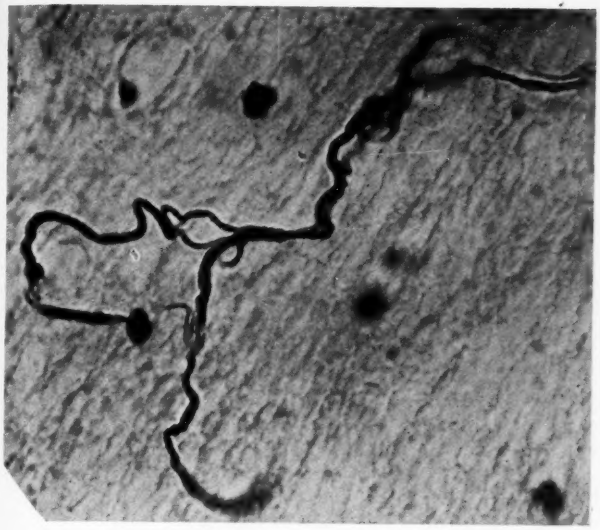


Fig. 1

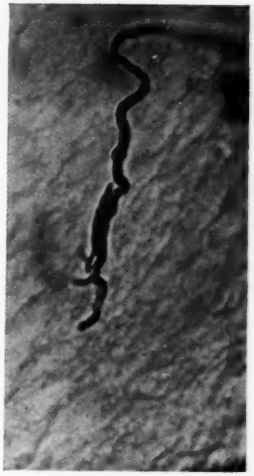
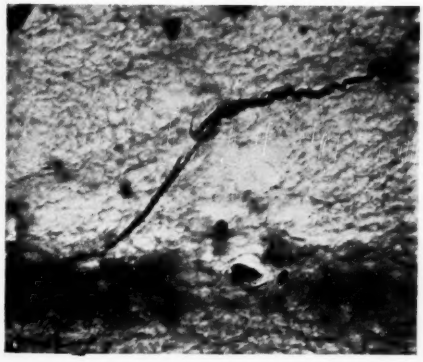


Fig. 2



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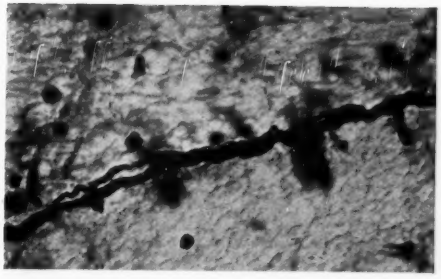

Fig. 4

Fig. 5

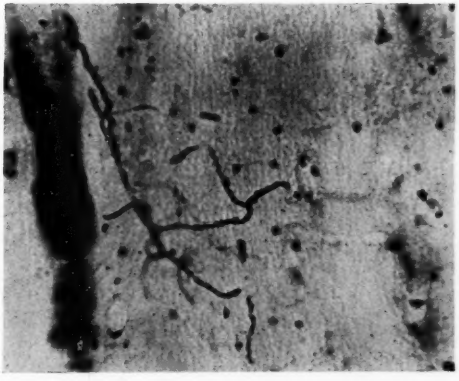

Fig. 6

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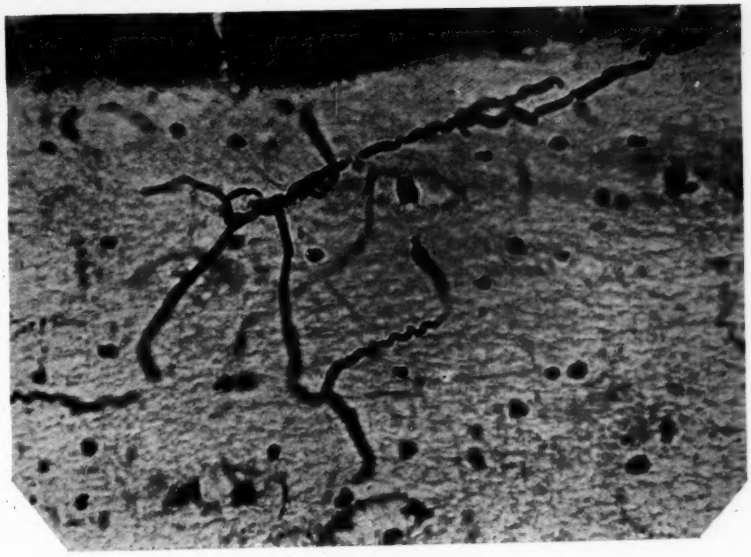

Fig. 1

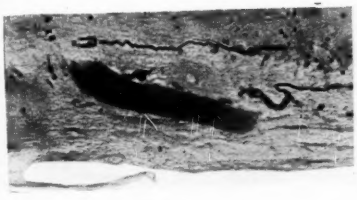

Fig. 2

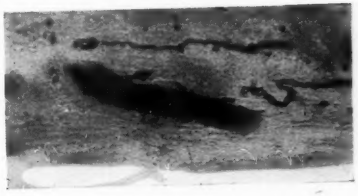

Fig. 3

Fig. 5

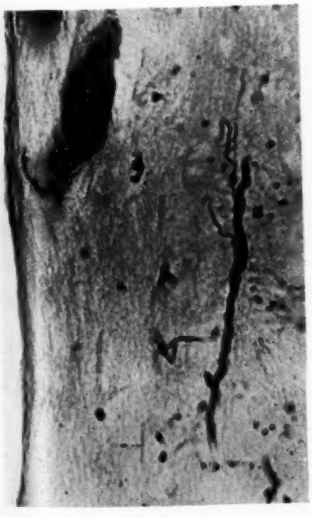

Fig. 4

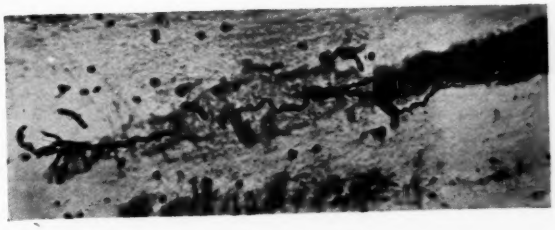

Fig. 6

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PLATE II

Fig. 1. Shows the histological characters of the nerve fibres. In the middle of the lower edge of the field will be seen the outline of a lacuna with the cell body and nucleus shrunken to the upper left side. The fibre coming down can be traced as far as the cell body. ×1190.

Figs. 2 and 3. Two views of the same field showing the entry of a Haversian canal and nerves from the periosteum. Fig. 2 is focused on a nerve entering the bone parallel to the canal, Fig. 3 on twigs applied to the vessels in it. ×306.

Fig. 4. Shows a small piece of nerve fibre applied to a blood vessel in the commencement of a Haversian canal. A larger nerve fibre is also seen entering the bone matrix well outside but parallel to the canal. ×588.

Fig. 5. This section is from the heavily impregnated side of the block. Shows Sharpey's fibres together with nerve fibres in the matrix. ×468.

Fig. 6. Shows nerve fibres lying in an obliquely cut Haversian canal. ×542.

CULTIVATION OF NERVE CELLS *IN VITRO* OVER A LONG PERIOD. SECOND NOTE

By H. MEYER and W. JABLONSKI

From the Institute of Anatomy, The University of Turin, Italy

In the *Rivista di Biologia*, vol. XXII (1937), we gave a report of the first results of a series of experiments on the cultivation of spinal ganglia of chicken embryos over a long period of time. The ganglia are cultivated in Carrel-flasks, type A, according to the technique developed by Fischer & Parker(1) for experiments on the differentiation of fibroblasts. For all details of technique, our first communication should be consulted.

The cultures of spinal ganglia which have survived longest have now been 19 weeks *in vitro*. Up to this date the cells seemed in good condition. Very clear neurofibrillae could be seen in a certain number of them during life without fixation and staining.

Some of the oldest cultures, one of them after 135 days' cultivation *in vitro*, have been fixed and stained. In the histological preparations the ganglion cells also show a perfectly sound state (see Figs. 5 and 6).

Frequently the cultures are spread over a wide area (about a third to half an inch), but the nerve cells are always limited to a small area in the middle of the culture, and in those cultures which have not been transplanted they are confined to a single stratum. No increase in the number of ganglia cells, and no emigration of these into the area of migration of the mesenchyme cells has been seen. In cultures which had been divided through the middle we noticed that, from the place where the group of ganglion cells had been bisected and in consequence immediately confined to the coagulum, no nerve fibres had been sent forth, nor did any ganglion cells emigrate into the coagulum; and yet the ganglion cells seemed normal and sound. Later on, examining the stained preparations, it became evident that the regenerated nerve fibres formed very often a thick ring around the explant. The nerve fibres rarely went beyond the limit between the centre and fresh coagulum; the outgrowing nerve fibres there often changed direction and returned to the centre.

These facts render the cultivation of ganglion cells over a long period, of course, very difficult. The cultures cannot be multiplied by dividing them. Till now it has only been possible to secure survival of some of our cultures. But we could not, as in the case of fibrocytes, obtain strains out of a small number of cultures. And till now, only a few out of the great number of spinal ganglia which we have explanted have survived *in vitro* for more than 2 months.

This behaviour of the cultures is perhaps determined by defects of our technique, but on the other hand may be due to the peculiarities of the nerve cells. In vertebrates, the cells of the primordia of the nervous system divide

only at an early embryonic period. The differentiated spinal ganglion cells of the chicken embryo which have been used in our experiments, do not divide in the organism. It is possible—and our studies seem to confirm it—that these cells do not regain the property of dividing even under the changed conditions of cultivation *in vitro*.

In many of our cultures the nerve cells degenerated. This degeneration might be various but in most it followed a uniform pattern.

Sometimes the cells would shrink and become more refractory; the structure of the cells became invisible; sometimes round very refractory parts of the cytoplasm detach themselves from cells which to all appearance are healthy. In other cultures, there appear in all cells granules, set in concentric circles, following the direction of the neurofibrillae.

In our cultures either the nerve cells degenerated together with the non-nervous cells (mesenchyme cells, sheath cells) or the non-nervous cells were in a good condition, when the nervous cells degenerated. In one case, an explant of the great vessels of another embryo had been added to a spinal ganglion. This time, the fibroblasts of the added tissue disintegrated, while the ganglion cells did not seem damaged. These became more visible because the covering fibroblasts vanished.

It is therefore evident that the connexions between the ganglion cells and the fibroblast—like cells in the explants of spinal ganglia—are closer than those between the spinal ganglion cells and the fibrocytes of another tissue.

Preparations fixed and stained after Cajal's method often resemble those of ganglia cultivated upon cover-slips. Here, too, the size of the cells differs considerably, the neurofibrillae are more or less fine, and more or less close together; without there being any evident connexion between the shape of the fibrillae and the external conditions.

The cells in the oldest of our fixed and stained cultures, which had been cultivated *in vitro* for 41, 48 and 135 days respectively, showed, however, very marked differences from the usual shape of ganglion cells *in vitro*. The outline of the cells in these cultures is not regularly oval or round as in nerve cells cultivated for a short time, but irregular, with lobes and excrescences, visible in living cultures too. These excrescences are bulky at first; in older cultures there appear fine and frequently very long excrescences, often ending with an intumescence. In other matters, too, nerve cells in older cultures differ from those which have been observed *in vitro* previously; in the optic section, their area shows an increase of three or four times; the network of the neurofibrillae is very fine, finer than usual in silver-preparations after the Cajal method.

It will be easier to understand these observations if we remember the conditions in the organism. Differentiated nerve cells of vertebrates do not divide. They follow the increase of the volume of the organism by increasing their own volume. In this, and in this only, consists the growth of nerve cells. So, in big vertebrates the volume of ganglion cells becomes very considerable.

In these forms (Homo, Equus, Testudo, Orthagoriscus), the borders of the cells are irregular with lobes and excrescences, or the cells are "windowed", two phenomena which become more accentuated with the age, the cells then assuming very complicated forms (Levi(2)). By this change of outline their surface increases, and more favourable conditions for the nutrition of the cells are attained than would have been possible in big cells with simple round periphery. Similar to these cells of big vertebrates are the cells described in this paper in cultures of spinal ganglia of the chicken embryo.

It seemed probable, *a priori*, that in the cultivated nerve cells too, the increase of surface area by lobes is the consequence of a growth of the cell-volume. Researches begun by Levi & Meyer, on cross-sections of cultures on cover-slides, here reported for the first time, demonstrated that the spinal ganglion cells *in vitro* at first are almost spherical; but later on they become somewhat flattened out, assuming an ellipsoid shape. In our old cultures with irregular-shaped, widely expanded cells, the numerical relation between plasma and nucleus, "Kernplasmarelation", in the optic section has undergone a change in favour of the plasma. The nucleus, situated in the midst of the cell, is probably flattened out to a lesser degree than the cytoplasm, especially in its peripheral parts. And only by this change in shape the "Kernplasmarelation" in the optic section has altered, the primary "Kern-plasmarelation" in reality being unaltered.

In our experiments, the ganglion cells did not reach the size of the ganglion cells of big vertebrates. However, according to the researches of Levi & Meyer, it seems that, even taking into consideration the flattening of the cells, the ganglion cells which have been cultivated for a long period, increase in volume and are bigger when compared with those which have been explanted but a very short time.

SUMMARY

1. The oldest of the spinal ganglia cultivated by us with the technique of Fischer & Parker for experiments on fibroblasts survived in a sound state for 19 weeks.

2. Till now no multiplication of ganglion cells has been observed. The ganglion cells do not migrate into the migration zone of the mesenchyme cells. In this, the cultivation of ganglion cells differs from the cultivation of other cell types. It was not possible, as it is with fibroblasts, to obtain a strain from a small number of cultures. It has only been possible to make a certain percentage of the explants survive.

3. The silver preparations of spinal ganglia cultivated *in vitro* up to 19 weeks showed that the ganglion cells and neurofibrillae are in an excellent condition. Some of them resemble the cultures on cover-slips cultivated over a short period. In most of the older cultures, however, the shape of the cells differs considerably: the periphery is irregular, they have lobes, and sometimes very long and fine excrescences. These cells bear a great resemblance to those of the spinal ganglia of the big vertebrates.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

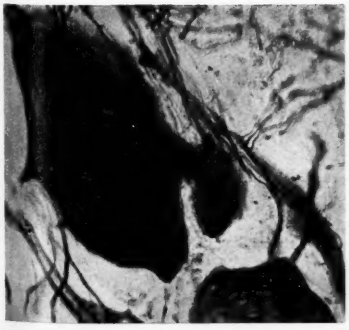

Fig. 5

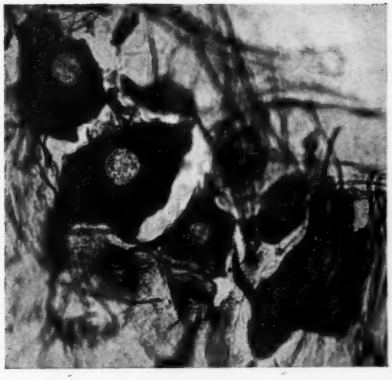

Fig. 6

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EXPLANATION OF PLATE I

Fig. 1. Part of culture of spinal ganglion of a chicken embryo of 10 days. Cultivated *in vitro* for 40 days. ×600.

Fig. 2. Part of culture of spinal ganglion of a chicken embryo of 11 days. Cultivated for 41 days *in vitro*. ×440.

Fig. 3. The same as Fig. 2.

Fig. 4. Part of culture of spinal ganglion of a chicken embryo of 14 days. Cultivated for 48 days *in vitro*. ×420.

Fig. 5. Part of culture of spinal ganglion of a chicken embryo of 12 days. Cultivated for 135 days *in vitro*. Plan section. ×670.

Fig. 6. The same as Fig. 5. ×500.

All figures are Photomicrographs of spinal ganglia of chicken embryos, cultivated *in vitro* and fixed and stained after Cajal's method.

THE TORUS MANDIBULARIS IN THE BUSHMAN

By M. R. DRENNAN

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The presence of one or other of the types of torus mandibularis in all of the Peking mandibles, and the great significance which Weidenreich (1936) attaches to this feature in these jaws, has revived interest in this peculiar variation of the human mandible. Before the discovery of Sinanthropus, investigators of the torus had noted that in certain groups of mankind, such as the Eskimos, Lapps, Ostiaks and Icelanders, its incidence was relatively high, whereas it only occurred sporadically in Europeans and American Indians, and it was quite unknown in Negro, Australian and Melanesian mandibles.

In spite of this indication that the torus mandibularis is essentially a racial characteristic, the majority of previous writers on this subject have attributed its presence to mechanical causes, regarding it as a hyperostotic reaction to the strains and stresses of chewing. This physiological explanation, however, is very unconvincing when one considers that the Australian and the Negro mandibles are put to very hard usage, and yet do not develop tori.

In his study of the Peking mandible Weidenreich (1936) has extended the survey to Chinese material, and he finds therein a considerable incidence of the torus. He also quotes figures to show that it occurs with considerable frequency in the Japanese. From his study of the data that he has collected he comes to the conclusion that the torus is a morphological rather than a physiological or a pathological character, and that the Mongolian races have a very considerable, if not quite a complete, monopoly of it. An important point which he stresses is that in all the groups in which the condition has been studied there is a very decided reduction in its incidence as we proceed from prehistoric and historic material to present-day man. All these facts give great significance to the occurrence of a torus in the Peking mandible, and Weidenreich seems justified in regarding its presence there as indicative of some genetic linkage between Peking man and the modern Mongolian.

This new conception of the morphological value of the torus has prompted me to record the extent of its incidence in the Bushman mandibles that I have at my disposal. The relative frequency of the occurrence of the torus in the Bushman raises again the very interesting issue as to whether he is not, after all, racially akin to the Mongolian. This theory has been mooted time and again, but in the absence of sufficient scientific data it has hitherto been largely discredited. In my opinion the evidence which I propose to submit goes a long way towards substantiating the idea.

In this connexion it is worth recalling that the early Dutch settlers at the Cape, who had ample experience of the peoples of the East, frequently referred to the aboriginals as "Chinese Hottentots". The features that suggested this appellation were no doubt the light yellow skin colour and the slanting set of the eyelids. The presence of the epicanthic fold has often given rise to comparisons with the Mongolian eye and suggested some racial affinity between the Bushman and the Chinese. The fact also that the Bushman is now generally accorded a cultural and physical relationship to the Aurignacians of Europe seems to me to bring an earlier connexion with more Northern races, such as the Eskimo, and with Asiatic races, such as the Chinese, within the range of possibility.

Of much greater significance, however, is Pijper's (1932) data concerning the blood groups of the Bushman. In this very important paper dealing with the serology of a fast-vanishing race the author states: "It is thus shown on purely serological grounds, without any further anthropological considerations, that the Bushmen are a race quite apart from, and having originally nothing to do with, any other African race or tribe." And when he comes to state the races with which the Bushman has the closest blood relationship, it is to the northern Icelanders and Eskimos that he appeals. The evidence which I now submit from the torus mandibularis, about the validity of which there does not appear to be any doubt, goes further to substantiate this thesis that the Bushman has some relationship with the Mongolian races and still farther back with *Sinanthropus*.

In Pl. I, A and B, I have illustrated two of the best examples of the torus mandibularis from my own collection of Bushman skulls. In Mandible A there are bilateral rounded tubercles situated in line with the second premolars, and about 12 mm. from the alveolar margin. The molar region of the internal alveolus is also slightly striated on both sides of this mandible. The perfect dentition and the bilateral symmetry of the tubercles completely exclude any pathological element in the causation, and the absence of undue wear of the teeth is, in my opinion, an argument against the theory that these hyperostoses are functional in origin.

In Mandible B there is a prominent tubercle located in line with the second left premolar, and about 8 mm. below the alveolar margin; whilst in front of it and lingual to the left canine there is a much smaller tubercle, situated 5 mm. from the alveolar border. In this mandible there are also striations lingual to the left molars. This formation, which is a very characteristic type of torus in other races, is not so well marked on the right side, where there is a more generalized hyperostosis, which may be partly pathological as the result of disease and subsequent loss of the molar teeth.

In my collection of twenty-eight Bushman mandibles there are nine examples of more or less well-defined tori of the tubercular type just described, giving an incidence of 32 per cent. Ten of the mandibles, that is to say 36 per cent of them, show striations in the molar region, and half of these striated

alveoli are in mandibles that also show a tubercular torus. Weidenreich maintains that these striations are a type of torus, so that, if these are included, the total incidence of torus mandibularis in these Bushmen is 50 per cent.

I have investigated the torus palatinus in this same group of Bushman skulls, and I find that it is present to a more or less marked extent in nine of them. This gives an incidence of 32 per cent, the same as for the tubercular type of torus mandibularis. All of these palatine tori are median in type, and several of them spread as diffuse thickenings over a considerable extent of the palate. Pl. I, C, shows one of the most marked of these tori, presenting a thickness of 1 cm. near the middle line. This particular torus palatinus was associated with a well-marked tubercular torus on the mandible. This high incidence of a palatine torus in these Bushmen is all the more noteworthy seeing that Deniker, as quoted by Middleton Shaw (1931), recorded a complete absence of this feature in twenty-seven Bushman skulls.

The degree of correlation between the mandibular and the palatine torus is a matter of considerable interest. I have found that the mandibular torus has a palatine torus associated with it in 66·7 per cent of instances. Although Hooton found a similar close relationship in the Eskimo, and Shreiner in the Lapps, Weidenreich is of opinion that there is no real correlation between the two formations. He bases his reasons for dissociating them on the difference in bony texture of these two tori, and on the fact that the torus palatinus occurs with very much greater frequency than the torus mandibularis in Italians, American Indians and in the Japanese. The torus palatinus may even occur in certain races that do not manifest a torus mandibularis. Thus Middleton Shaw (1931) has found an incidence of torus palatinus to the extent of 7 per cent in 132 Bantu skulls, not one of which showed a torus mandibularis. Future studies may unravel this curious relationship, but in the meantime it is quite clear that there is no necessary connexion between these two formations.

I have examined ten Hottentot skulls for the incidence of both tori, and I find that there is only one specimen in this group showing a slight tubercular torus mandibularis associated with a faint torus palatinus. This lesser incidence of 10 per cent is consistent with the fact that the Hottentot, although closely related to the Bushman, has also a considerable admixture of Hamitic and Negro blood.

Five young Bushman skulls and five young Hottentots in my collection show no trace of any of the torus formations.

My material is drawn in equal numbers from the coastal belt and from the inland parts of South Africa, but there is no significant difference in the incidence of the tori in the two groups.

INCIDENCE IN A BUSHMAN TRIBE

In a previous study of the dentition of a Bushman tribe (1929), based on an examination of material exhumed from "small-pox" graves situated near the town of Colesberg, I reported the presence of a well-marked torus mandibularis in one of the mandibles. This specimen is shown in Pl. I, D, and it presents a huge torus situated lingual to the canines and premolars on each side, with its maximum convexity 12 mm. distant from the alveolar margin. Through the break in the outer alveolus I was able to determine that this torus consists entirely of compact bone, measuring 1 cm. in thickness. I therefore agree with Weidenreich that these tori show no indication that they have been thrown up to meet some new strain or that they could have served this purpose. Rather would they seem to be the remains of some of the old and now useless scaffolding of the Simian jaw that has for some unaccountable reason not been completely absorbed in these human mandibles. In the present group of jaws there is ample evidence that they are only in a transitional stage from the ape-like form.

Through the courtesy of Dr L. Gill, Director of the South African Museum, I have been able to make a closer study of these fifty Bushman skulls. The torus mandibularis occurs in a tubercular form in twelve of them, giving an incidence of 24 per cent. With the exception, however, of the one which I have figured, they are all very poorly marked and hardly noticeable. Only three of the mandibles show faint striations, and these occur along with anterior tubercles. A torus palatinus occurs in only 6 per cent of these skulls, and in no instance is it well developed.

These Bushmen were buried in 1866, and I regard them as being much more recent than the majority of my own specimens, many of which must be prehistoric in the South African sense of this word. That they were very typical Bushmen is brought out by Slome's investigation of their skeletal parts. It would seem, therefore, that with their advance in civilization these Bushmen have shown a decided tendency to lose their tori, a phenomenon that has occurred in all the other racial groups in which the torus has been studied.

In addition to the racial incidence of these tori there is some evidence from the literature regarding them that they are also familial, several examples of occurrence in different members of the same family having been recorded. I think we are justified therefore in regarding them as a manifestation of a special genetic constitution. In the two sets of Bushman skulls that I have studied it is impossible to say whether the reduction in the tori in the more civilized group reflects a genotypic evolutionary advance or whether the genetic basis is unaltered and the reduction is merely an altered phenotypic expression of this as the result of a change in dietetic habits. I am inclined to think that in the short period of time that has elapsed between the prehistoric and the more modern Bushman there has probably been no genotypic change and that the diminution in the tori has been due to functional causes. One must

accordingly admit that conversely these tori may manifest themselves as a response to some functional stimulus, but in my opinion they can only appear in individuals with the appropriate genetic background. A concession to this extent does not impair in any way my argument for their essential genetic nature nor does it affect the validity of the thesis that I have based thereon, namely that there is a real kinship between the Bushman and the Mongolian.

CONCLUSION

In this paper I have described the incidence of the torus mandibularis and of the torus palatinus in two groups of Bushman skulls, and I have advanced arguments therefrom to show that the Bushman race has undoubted physical affinities with the Mongolian races.

My thanks are due to Mr A. A. Lamb, my technical assistant, for providing the photographic illustrations.

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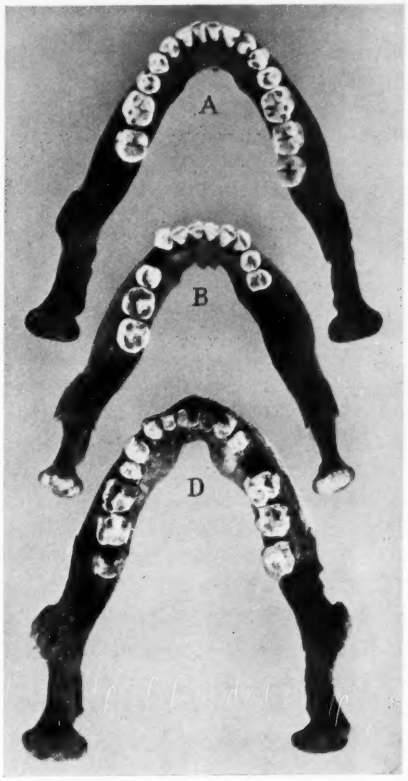
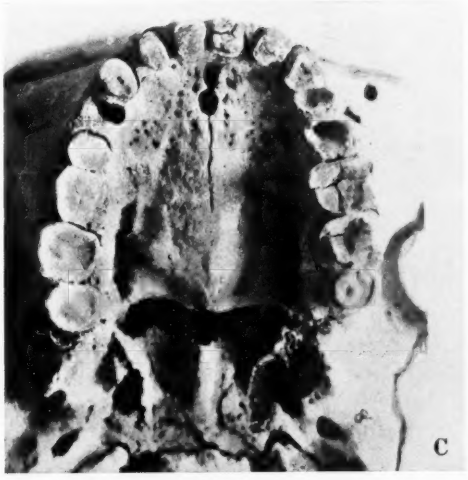
EXPLANATION OF PLATE I

A and B. Bushman mandibles showing different types of tubercular torus mandibularis. C. Torus palatinus on the palate of a Bushman skull. D. Mandible of a recent Bushman showing the torus mandibularis.





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THE SUPRARENALS OF THE LARGER FELIDAE

By W. C. OSMAN HILL, M.D.
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INTRODUCTION

In 1930 I mentioned, in dealing with the suprarenals of young Carnivora, that I had found in a new-born leopard that the suprarenal gland was exceptionally large like that in new-born Primates. Measurements were given and the organs figured and compared with those of new-born kittens and other Carnivora, but no special discussion was entered into on the strength of a single specimen. Recently I have been able to complete an examination of a larger series of stages in the leopard, and to compare these with some stages in the lion and tiger. My results are here outlined and the suprarenals of these animals are compared with those of domestic cats.

MATERIAL AND METHODS

The material examined consists of seven stages in the life history of the leopard, augmented by one stage from the tiger and five from the lion. These have been compared with a series from the postnatal period in the domestic cat. Linear measurements of the fresh suprarenals and kidneys were followed up by taking the weights of the organs after fixation.

Fixation in all instances was in equal parts Müller's fluid and 10 per cent formalin. Slices were taken from the suprarenals and cut with the freezing microtome at 15μ, care being taken to make sections from strictly comparable parts of the glands in the different specimens. The frozen sections were stained in haematoxylin and Sudan III. In most cases ordinary paraffin sections were also examined after staining in haematoxylin and eosin.

I. GROWTH CHANGES IN THE SUPRARENAL OF THE LEOPARD

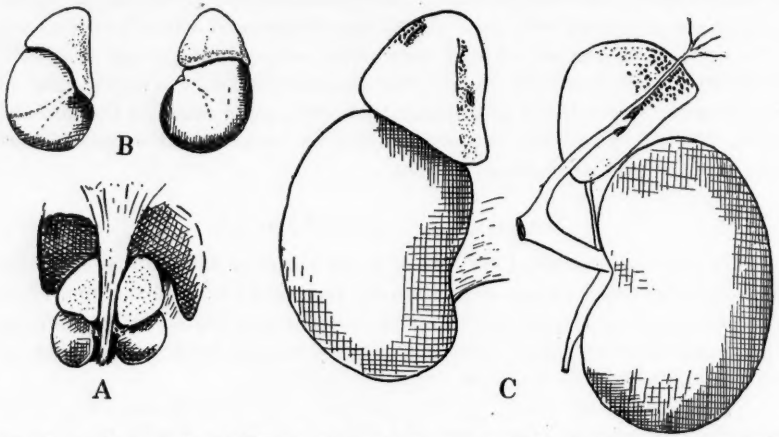
The following series of stages in the leopard have been examined:

(1) Mid-term foetus, male, C.R. 72 mm.
(2) Older foetus, male, C.R. 150 mm.
(3) Full term foetus, female, C.R. 210 mm.
(4) New-born cub, male (previously reported on).
(5) Cub, male, a few months old, length 850 mm.
(6) Cub, half-grown.
(7) An adult leopardess.

The suprarenals and kidneys of some of these are illustrated in Fig. 1. Measurements are given in the accompanying Table I; and in Table II the weights of the suprarenals are compared with the weights of the kidneys and with the total body weight.

The figures and tables indicate that a similar change in size relations to neighbouring organs and to the body as a whole occurs in the developing leopard's suprarenal as is found in young Primates, and that these changes occur at a corresponding age period.

At mid-term the suprarenals in this animal are larger than the kidneys and are actually double the latter in weight. In shape they are conical, but present no dorso-ventral flattening such as is characteristic of the older gland. The surface, moreover, is quite smooth. With advancing age the suprarenal, though



Text-fig. 1. Suprarenals and kidneys of a series of young leopards. A. Suprarenals and neighbouring organs of a mid-term foetus. Dorsal aspect. B. Kidneys and suprarenals of a more advanced foetus. Ventral aspect. C. Kidneys and suprarenals of a young cub, a few months old. Ventral aspect.

growing larger, does so to a less degree than the kidneys and other organs, so that it appears relatively smaller. This process commences in prenatal life, but is continued for some considerable time after birth.

In section the suprarenal at mid-term presents an oval outline. Microscopically it consists of cortical tissue. The capsule is well developed, and the cells beneath it are small, but not arranged in glomerular formation as yet. The rest of the gland is composed of larger, clearer cells, with no special arrangement.

In two later prenatal stages but little change is evident. The gland still remains large when compared with the kidney, but there has been a relative decrease as compared with the mid-term stage (see Table I). The gland remains smooth and rounded, but there is definite advance in internal structure. To the

naked eye the cut surface remains homogeneous, but microscopically, there is a distinct differentiation between the surface cells and the main mass of the gland. The surface cells are small, stain deeply, and have condensed nuclei. They tend to lie in columns, the tops of which tend to fuse to form rounded cell-bunches, but show no glomerular arrangement. Between the columns vessels

Table I. *Measurements of the suprarenals and kidneys of the larger Felidae*

Specimens examined	Left suprarenal Wt. g.	Ht. mm.	Br. mm.	Th. mm.	Right suprarenal Wt. g.	Ht. mm.	Br. mm.	Th. mm.
1. Leopard foetus ♂, C.R. 72 mm., wt. 20 g.	0·1	9	7	4	0·1	7	7	5
2. Leopard foetus ♂, C.R. 150 mm., wt. 315 g.	0·52	11·5	13	6	—	9	13	7
3. Leopard foetus (full term) ♀, C.R. 210 mm., wt. 475 g.	0·3	12	13	5	—	—	—	—
4. Leopard, new-born ♂	—	12	9	7	—	—	—	—
5. Leopard cub, wt. 6802 g.	3	30	29	9	2	32	25	9
6. Leopard cub ♂, wt. 16,325 g.	3	19	34	5	4	19	30	5·5
7. Leopard, adult ♀, wt. 25,396 g.	4·25	22	35	9	3·7	24	24	9
8. Tiger, half-grown ♀	4·2	44	20	10	5	52	31	6
9. Lion, 6 days old ♂, wt. 1230 g.	0·65	14	15	5	0·85	17	14	6·5
10. Lion, 22 days old ♂	0·56	10	16	5	0·65	16	12	6
11. Lion, 35 days old ♀	0·47	11·5	13	4	0·44	18	12	5
12. Lion, half-grown ♂, wt. 27,210 g.	4·05	28	28	8	4·4	21	37	13
13. Lioness, adult	5·15	35	32	5	6·9	39	38	6

Specimens examined	Left kidney Wt. g.	L. mm.	Br. mm.	Th. mm.	Right kidney Wt. g.	L. mm.	Br. mm.	Th. mm.
1. Leopard foetus ♂, C.R. 72 mm., wt. 20 g.	0·05	7	5	4·5	0·05	7	4·5	4·5
2. Leopard foetus ♂, C.R. 150 mm., wt. 315 g.	1·95	19	15	10	1·45	18	14	10
3. Leopard foetus (full term) ♀, C.R. 210 mm., wt. 475 g.	2·29	18	16	12·5	2·5	21	19	13
4. Leopard, new-born ♂	—	30	20·25	15	—	—	—	—
5. Leopard cub, wt. 6802 g.	42·5	62	38	30	39	54	36	32
6. Leopard cub ♂, wt. 16,325 g.	65	63	43	35	70	61	47	32
7. Leopard, adult ♀, wt. 25,396 g.	56·2	60	46	35	55·1	56	46	36
8. Tiger, half-grown ♀	73	78	42	34	75	79	44	36
9. Lion, 6 days old ♂, wt. 1230 g.	10	34	23	20	10	34	21	23
10. Lion, 22 days old ♂	11·6	31	25	20	12·3	40	22	24
11. Lion, 35 days old ♀	13·46	40	27	23	13·12	39	28	21
12. Lion, half-grown ♂, wt. 27,210 g.	140	84	60	50	145	91	57	48
13. Lioness, adult	210	100	70	55	240	100	75	55

can be seen. The rest of the gland remains as before, but the cells tend to become arranged radially around the central venous sinus. No further change occurs structurally till after birth, apart from a slight increase in vascularity.

Immediately after birth the suprarenal has commenced a process of dorso-ventral flattening, due to removal of some of the deeper cortex. Microscopic examination shows this to be caused by a degeneration of some of the deepest part of the cortex, next to the central vein. Meantime islets of chromaffin tissue have become evident in the deeper part of the undegenerated cortex.

The subcapsular cortex has by this time taken on a definite glomerular formation.

No postnatal stage in the leopard was available between the new-born state and the cub (No. 5) a few months old. It is during this period that the most drastic changes in suprarenal form and structure take place, as evidenced by the stages studied in the lion and described below. In the youngest leopard cub, therefore, considerable differences are noticeable from the new-born stage. Though the gland is still relatively large compared with that of the adult, it is relatively very much smaller than in the new-born animal. It weighs only 1/16th of the kidney weight, whilst the ratio of the latter to the total body weight has now become tolerably stable. The suprarenal is now a flattened object, and this flattening proceeds rapidly and reaches its maximum in the

Table II. *Proportions of body weight to weights of suprarenals and kidneys in the larger Felidae*

Specimens examined	Body wt. g.	Proportion of suprarenal wt. in kidney wt.	Proportion of suprarenal wt. in body wt.	Proportion of kidney wt. in body wt.
1. Leopard foetus ♂, C.R. 72 mm.	20	2	1/100	1/200
2. Leopard foetus ♂, C.R. 150 mm.	315	1/3	1/300	1/100
3. Leopard foetus (full term) ♀, C.R. 210 mm.	475	1/8	1/791	1/100
4. Leopard, new-born ♂	—	—	—	—
5. Leopard cub	6,802	1/16·3	1/1360	1/83
6. Leopard cub ♂	16,325	1/19	1/2332	1/121
7. Leopard, adult ♀	25,396	1/14	1/3174	1/227
8. Tiger, half-grown ♀	—	1/16	—	—
9. Lion, 6 days old ♂	1,230	1/13·3	1/820	1/61·5
10. Lion, 22 days old ♂	1,350	1/19	1/1115	1/56
11. Lion, 35 days old ♀	1,574	1/29	1/1729	1/59
12. Lion, half-grown ♂	27,210	1/33	1/3220	1/95
13. Lioness, adult	—	1/37	—	—

next older cub (No. 6). In section the gland gradually assumes a trifoliate outline. The cut surface presents a superficial zone of pale, firm, yellowish cortex followed by a more bulky dark, friable mass containing the main vessels. The gland consists of two plates of cortex thus constructed, and between them lies the central vein and a small modicum of medullary tissue. Microscopically each cortical plate is composed of a glomerular zone, a medium-sized fasciculate zone and a zone where some slight intercommunication of neighbouring cell columns causes a reticular appearance. The last-mentioned part of the cortex is more vascular than the rest. Medullary cells form only a thin coating on the walls of the central venous sinus and its immediate tributaries. The two cortical plates are thinner in the older cub than in the younger, chiefly on account of the lesser depth of the fasciculate layer. Reticular formation is present, and remains very vascular. At this stage also a zone of vascular connective tissue separates the cortex sharply from the medulla.

The adult condition was studied in a leopardess, 9 years old. In weight the glands of this animal are only a few milligrams heavier than in the last-

mentioned cub, and hence they form a considerably less proportion of the total body weight. The glands are now much broader and higher relative to their thickness, the two sides differing somewhat in detail. The surface of each gland is now wrinkled, as in adult Primates, though not to such a marked extent as in that order. The cortical plates have much the same structure microscopically as in the last stage, but they are thicker, due to an increase in length of the columns in the fasciculate zone. These columns show little or no interlacement, except at their central ends where they gradually give place to a true, but narrow reticular zone. A stout band of comparatively avascular fibrous tissue separates each cortical plate sharply from the medulla. It may be regarded as a scar tissue. The medulla is greater in quantity than in the immature animal.

II. GROWTH CHANGES IN THE SUPRARENALS OF LIONS AND TIGERS

No prenatal stages have been examined in Felidae other than the leopard, but from a study of the series of new-born lions described below it may be assumed that the suprarenal, in prenatal life, has a similar history to that described for the leopard.

The following postnatal stages have been examined in the lion:

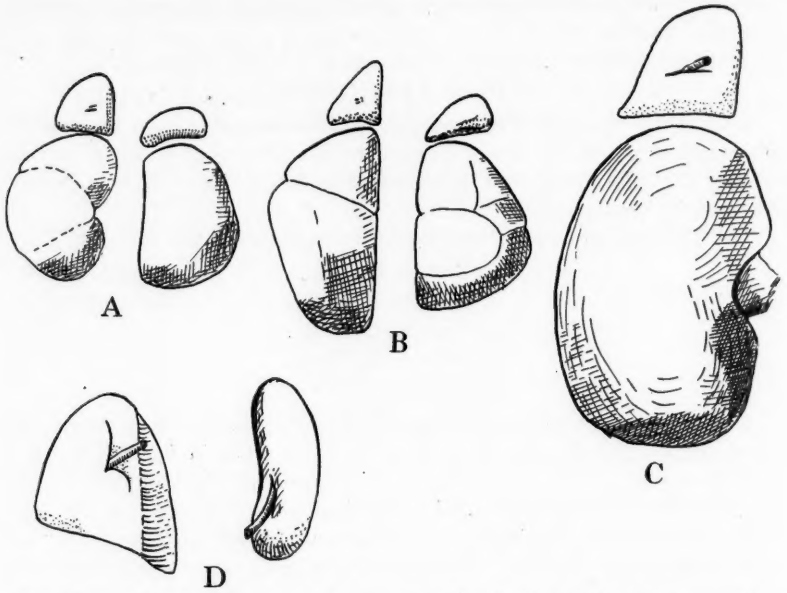
(1) Cub, 6 days old.
(2) Cub, 22 days old.
(3) Cub, 35 days old.
(4) Half-grown lion.
(5) Adult lioness.

In addition to the above one stage has been examined in the tiger—a half-grown female. The measurements of all these are given in Table I, whilst in Table II the ratios between suprarenal weight, kidney weight and body weight are given. The tiger was emaciated so that its body weight was not considered worth recording, but the suprarenal-kidney ratio is probably reliable, for it is quite comparable with that of the older leopard cub and of the older lion cubs.

In the 6 days old lion the suprarenals are full and rounded, with smooth surfaces and no sharp borders. The two glands differ in shape from those of the leopard. In section each gland has an elongated oval outline and is composed of two cortical plates. What looks like medulla on the chromic fixed specimen is the deeper part of the cortex. No medulla is visible to the naked eye at this stage. Under the microscope the connective tissue capsule is seen to be very cellular. The zona glomerulosa is comparatively narrow and consists of the usual small cells. They are smaller in the lion than the leopard. Deep to this zone lies a fasciculate stratum, composed of larger cells of granular appearance with vesicular nuclei. The deeper half of the cortex is composed of continuations of the above columns, but the cells are smaller and the columns are forced apart by the engorgement of the intervening blood spaces. The deepest moiety consists of similar cells with a more reticular arrangement, and with engorged

blood spaces in the meshes. These last-mentioned cells are continued right on to the walls of the central venous channels. Chromaffin tissue is represented by a few scattered clumps of opaque cells—larger than their cortical neighbours.

In the next lion cub, approximately 2 weeks older than the last, the suprarenals have similar shape, but are appreciably smaller in size. Microscopically considerable changes have supervened. Pl. I, fig. 2 shows a section through the tip of one of the triradii of which each gland is composed. The capsule and zona glomerulosa are as in the last stage. The true fasciculate zone is also similar, but the columns are somewhat shorter. Deep to this occurs a haemorrhagic



Text-fig. 2. Suprarenals, with kidneys, of a series of young lions and a tiger. A. Suprarenals and kidneys of a 6-day-old male lion cub (*Panthera leo*). B. The same of a 22-day-old lion cub (*P. leo*). C. Right kidney and suprarenal of a half-grown male lion (*P. leo barbarus*). D. The right and left suprarenals of a half-grown tigress (*P. tigris sumatrae*).

stratum representing the more irregularly arranged deep cortex of the 1 week stage. The vascular spaces are now further dilated and heavily engorged with blood. They are irregular in outline and freely intercommunicate. In parts the engorgement spreads radially into the deeper portion of the true fasciculate zone. The cellular elements between the dilated blood spaces are compressed and many have broken up and appear to be represented by large fat droplets. The centre of the gland is occupied by loose connective tissue of embryonic type, containing some sinusoids and some isolated clumps of medullary tissue.

These clumps are larger than in the last stage and seem to be due to fusion of the smaller clumps.

The suprarenal of the third lion cub illustrates the completion of the most dramatic episode in the cortical changes. In this stage, approximately 2 weeks later than the preceding, the gland exhibits an even greater amount of shrinkage. The organ is now flattened dorso-ventrally, due to the increased thinning of the two component cortical plates, and also on account of the absence of a macroscopic medullary lamina between them (Pl. I, fig. 3). Capsule and zona glomerulosa are unchanged, but the zona fasciculata consists of shorter columns, and its deepest moiety is now represented by a much thinner haemorrhagic stratum. The cells of this region are completely degenerated. Between the two cortical plates is a mass of cellular connective tissue, containing large, comparatively empty sinusoids. A few clumps of chromaffin cells occur as in the last stage.

In the half-grown lion the medulla has increased in size sufficiently to be visible to the naked eye, but is not yet as condensed as in the adult. The cell clumps are separated by a stroma of contracted, reticular connective tissue. This is comparatively free from chromaffin cells in certain places, such as the cortico-medullary boundary and the apices of the radii. It is obviously the descendant of the looser connective tissue of the neonatal period. The cortex differs chiefly from that of the neonatal suprarenal in the great increase in length of the columns composing the true fasciculate zone. The deep limits of this are somewhat irregular, but the above-mentioned connective tissue band sharply demarcates it from the medulla. There is no reticular zone, though column formation is not as perfect in the deepest layers of the fasciculate zone as elsewhere. The cells of this region further differ in their smaller size and degenerate appearance. This region of the cortex is evidently yet unstable and some change of a transitory nature is apparently still in progress. The vessels of this area too are engorged, though they are much smaller than in the neonatus. The suprarenals of the half-grown tigress agree in all essential particulars with those of the half-grown lion. The cortical plates are a shade thicker (2 mm.), whilst the medulla appears to the naked eye as a thin dark strip in sections of chromic fixed material. Microscopically the cortex is similar to that of the half-grown lion, but the zona fasciculata consists of longer columns which run parallel and relatively straight as far as the cortico-medullary fibrous lamina.

The suprarenals of the adult lioness agree in most particulars with those of the old leopardess. The zona fasciculata consists of similar long columns, but the zona reticularis is broader and its cells are clumped together to form a more solid structure than in the leopard. A broad band of fibrous tissue separates the reticularis from the medulla. The latter is similar in quantity to that of the old leopardess.

III. THE SUPRARENALS OF DOMESTIC CATS

It is not the intention in the present contribution to give a detailed account of the life history of the suprarenal glands in the lesser felines. This is a large problem and needs much material for its full elucidation. So far no foetal stages have been examined; hence it is not possible to state definitely that changes similar to those discussed above in the larger members of the cat tribe do not positively occur during that period. But from the stages that are known in the domestic cat, it is very evident that, even if some such change does occur,

Table III. *Measurements of the suprarenals and kidneys in domestic cats*

	Left suprarenal				Right suprarenal			
Specimens examined	Wt. g.	Ht. mm.	Br. mm.	Th. mm.	Wt. g.	Ht. mm.	Br. mm.	Th. mm.
1. Average of 5 kittens new-born, wt. 345 g. (3 ♂♂, 2 ♀♀)	0·05	6·8	5	2·8	0·05	7·3	4·7	3
2. Juvenile ♂ still at the breast, wt. 463 g.	0·05	7	5	3	0·04	7	4	3
3. Juvenile ♂	—	8	5	5	—	9	5	3
4. Juvenile ♂	0·15	8	6	6	0·10	9	6	5
5. Adult ♀ (lactating), wt. 2875 g.	0·2	12	6·5	5	0·2	11	8	5
6. Adult ♀, wt. 3083 g.	0·22	7	10	5	0·20	7	13	6
7. Adult ♀, wt. 3854 g.	0·25	6	12	5	0·24	7	11	6
8. Adult ♂ (entire)	0·22	9	8	5	0·22	14	7	4

	Left kidney				Right kidney			
Specimens examined	Wt. g.	L. mm.	Br. mm.	Th. mm.	Wt. g.	L. mm.	Br. mm.	Th. mm.
1. Average of 5 kittens new-born, wt. 345 g. (3 ♂♂, 2 ♀♀)	2·44	23·4	15·3	12·4	2·55	23·6	15	12·8
2. Juvenile ♂ still at the breast, wt. 463 g.	3·1	25·5	18	12	2·9	24	18	12·5
3. Juvenile ♂	—	28	19	15	—	27	18	15
4. Juvenile ♂	4·2	27	17	15	4·3	28	20	15
5. Adult ♀ (lactating), wt. 2875 g.	9·3	35	26	21	9·5	35·5	26	19
6. Adult ♀, wt. 3083 g.	10·2	37	26	18	9·6	37	24	18
7. Adult ♀, wt. 3854 g.	18·2	42	31	26	17·27	42	32	22·5
8. Adult ♂ (entire)	16·25	43	33	22	17·79	45	32	23

it certainly does not take place at the same age period. For this reason a brief statement of the size, shape and structural appearances of the suprarenals of new-born kittens and of adult cats is necessary.

In the accompanying table (III) are given the measurements of the suprarenal glands and kidneys of a number of cats at various ages from birth onwards. In the following table (IV) the weight ratios of the suprarenals to the kidneys and to the total body weight are given. These tables are to be compared with Tables I and II respectively.

From Tables III and IV it is manifest that the suprarenals of newly born kittens are vastly different from those of lions and leopards of corresponding age. Mere superficial examination of the organs whilst still *in situ* is sufficient to convince the observer of this fact. The suprarenals of newly born kittens

form approximately only 1/50 of the weight of the kidneys, and only 1/3450 of the total body weight. Advancing age does not materially alter the former, but there is a decrease in the latter ratio.

A study of the structure of the gland in cats any time after birth gives the clue to this difference in the weight ratios when compared with those of the larger felines. The suprarenal gland of the new-born kitten is evenly rounded and smooth. In section it presents a rather squat oval outline; and the cut surface presents for inspection to the naked eye both cortex and medulla, each sharply defined from the other. Microscopically the definition is not quite so

Table IV. *Proportions of body weight to weights of suprarenals and kidneys in domestic cats*

Specimens examined	Body wt. g.	Proportion of suprarenal wt. in kidney wt.	Proportion of suprarenal wt. in body wt.	Proportion of kidney wt. in body wt.
1. New-born kittens. Average of 5	345	1/49	1/3450	1/69
2. Juvenile ♂	463	1/60	1/4630	1/77
3. Juvenile ♂	—	—	—	—
4. Juvenile ♂	—	1/34	—	—
5. Adult ♀ (lactating)	2875	1/47	1/7187	1/153
6 Adult ♀	3083	1/47	1/7340	1/155
7. Adult ♀	3854	1/70	1/7708	1/108
8. Adult ♂	—	—	—	—

sharp, but it is much sharper than in half-grown large felines. There is no suggestion of a fibrous tissue layer between cortex and medulla. The deeper cells of the cortex abut against the most superficial medullary cells. The cortex consists of zona glomerulosa and zona fasciculata only, the latter quite thin. The fasciculate zone stains heavily with Sudan III throughout. Mitotic figures are numerous among the cortical cells.

The gland of an adult cat differs little from this. It is a much larger organ, and is more rounded, so much so as to be almost circular in outline when sectioned. Medulla comes to the surface at the hilum, but is covered everywhere else with a very thick cortical layer—at least four times as thick as that of the new-born kitten. The cortex is made up of a zona glomerulosa and a zona fasciculata only. The former resembles that of a new-born, but the latter is responsible for the great increase in cortical thickness, due to the enormous elongation of its component columns. These resemble those of the adolescent tiger in being relatively straight and not inclined to interlace with their neighbours. The cortico-medullary boundary is sharp but presents no fibrous stratum. The medulla is greater in quantity than in the new-born. Its cells are arranged in strands whose direction is towards the hilum, and thus at right angles to the columns of the cortical cells in the greater part of the gland. Between the strands lie the usual sinusoids.

DISCUSSION

Throughout the large mass of literature on the subject no reference is made to any cortical change comparable with that here described in the larger Felidae, except in Primates. It is only in the Primates that a hypertrophied cortex is known to occur during foetal life. In all other mammals so far studied the suprarenal cortex in late foetal and early postnatal life differs but little from the adult condition.

The findings in the large felines, whose suprarenals have been described above, are therefore somewhat unexpected. There is no doubt that the changes mentioned are on a par with those occurring in foetal and neonatal Primates. Although lack of material has prevented my following up the history of the suprarenal in any one species, it is fairly obvious that the stages described in the leopard and the lion can be taken as representing a process that occurs in both species, whilst the single case of the tiger presents no feature on which the same processes could be denied to occur in that animal also. Assuming this to be true, the history of the suprarenal gland in all these large Felidae may be summarized thus:

During foetal life the medulla is in a rudimentary condition, being limited to a few small clumps of chromaffin cells lying in the centre of the suprarenal, in the neighbourhood of the central vein and its tributaries. Cortex, on the other hand, is in a hypertrophic state. At mid-term the cortex is irregularly arranged and homogeneous throughout. As full term approaches the zona glomerulosa is differentiated and the peripheral portion of the remaining cortex assumes a columnar formation. The deepest cortex remains irregular or at most adopts a reticular formation. Even before birth a process of reduction in the thickness of the cortex commences. This process affects first the deepest portion of the tissue. Immediately after birth the reduction of deep cortex occurs more rapidly and is associated with curious vascular phenomena and with increase in depth of the zona fasciculata. By the end of the first week of postnatal life wholesale removal of the deeper half of the original cortex is well under way. In another 2 weeks the deeper half of the cortex is distinctly degenerate and in a further 2 weeks it is almost gone. Its place is taken by a thin zone of engorged sinusoids and deep to these a mass of loose connective tissue containing islands of medulla, formed by the fusion of the pre-existing smaller clumps of chromaffin. As cubhood proceeds some further removal of deep cortex is brought about, and for a time the layer of engorged sinusoids persists. Meanwhile the zona fasciculata grows in depth by the increase in the length of its component columns in a central direction.

Accompanying the cortical changes there is a growth in the medulla and, as each clump of cells enlarges, the masses fuse to form a more continuous medullary tissue. In this manner the intervening connective tissue is compressed into a supporting network whilst at the cortico-medullary boundary a thicker stratum is left representing the scar of the degenerated deep cortex.

A further, and perhaps still more unexpected, result of the present investigation is the striking difference shown between the condition at birth of the suprarenal in the large felines and that of the domestic cat, and probably therefore of all the smaller felines. The cat at birth, as shown above, has a small suprarenal already composed of a typical mammalian type of cortex, and a medulla that is sufficiently advanced to be visible to the naked eye. The cat further agrees with many other mammals, such as the guinea-pig (Elliot & Tuckett, 1906) in having a large postnatal increase in the depth of the cortex. There is undoubtedly considerable postnatal increase in the depth of the zona fasciculata in the large felines too, but this is not so disproportionate when compared with the other components of the gland as it is in the domestic cat.

Finally, it may be remarked that the differences in the history of the suprarenal glands of the large and the small Felidae is a further argument in favour of their recent separation by Pocock (1930) from the genus *Felis*. On the strength of the anatomical differences in their hyoid apparatus (first pointed out by Owen (1834)) Pocock placed the lions, tigers, leopards and ounces in the genus *Panthera*. I consider this arrangement should be maintained in view of their suprarenal differences.

SUMMARY AND CONCLUSIONS

1. Changes in the size, form and structure of the suprarenal glands have been described in a series of leopards, lions, and a tiger at progressive stages in the life history.

2. The above changes are essentially similar to those already known to occur in Primates, but not known in any other mammals.

3. The changes referred to depend on the presence, during foetal life, of a hypertrophied cortex, and the removal of this excessive tissue during the neonatal period.

4. The cortical changes are associated with alterations in the quantity and distribution of the chromaffin tissue. The latter is not present in sufficient quantity in foetal and new-born animals to be visible to the naked eye.

5. The state of the suprarenal in new-born and adult domestic cats has been briefly referred to for comparison with the conditions found in the large Felidae.

6. It has been shown that no changes comparable with those described for the large Felidae occur in the domestic cats, the cortex at birth showing no degenerative phenomena and the medulla being already visible to the naked eye. Postnatal growth is accompanied by an increase in the amount of cortex rather than the reverse.

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EXPLANATION OF PLATE I

Photomicrographs of four stages in the postnatal development of the suprarenal gland of the lion (*Panthera leo*).

Fig. 1. Transverse section of the suprarenal at 6 days after birth.
Fig. 2. The same at 22 days.
Fig. 3. The same at 35 days.
Fig. 4. The same in a half-grown male Barbary lion (*P. leo barbarus*).

ABBREVIATIONS

b.v. blood vessels.
ch. chromaffin tissue.
e. embryonic connective tissue.
z.f. zona fasciculata.
c. capsule.
c.v. central vein.
f.c. foetal cortex.
z.g. zona glomerulosa.

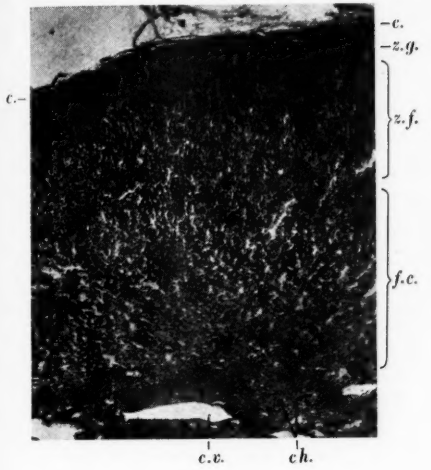

Fig. 1

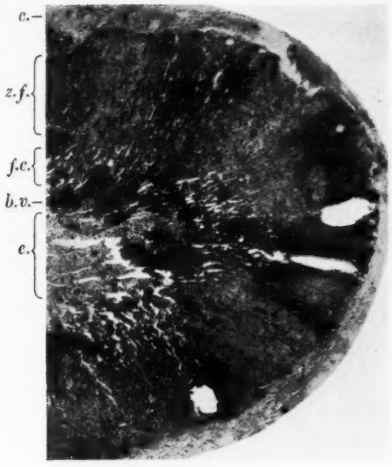

Fig. 2

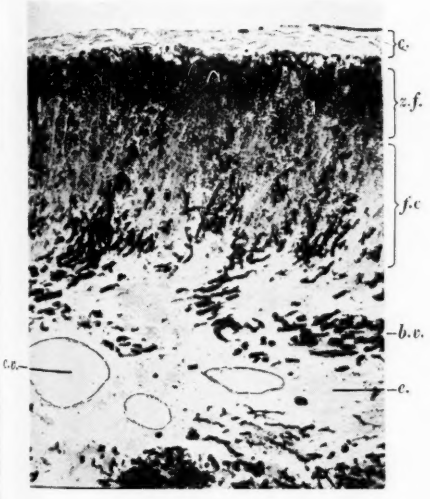

Fig. 3

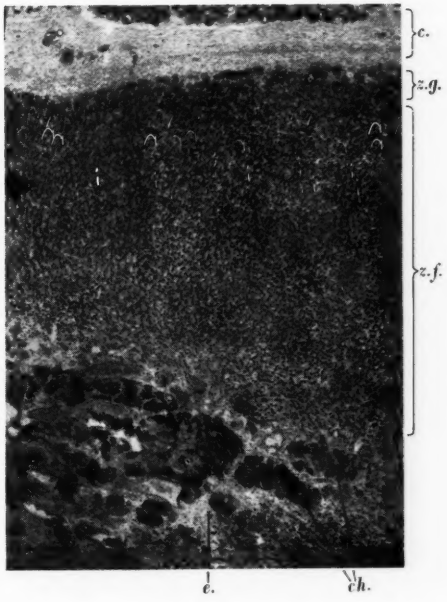

Fig. 4

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CONGENITAL UROGENITAL ANOMALIES IN RATS INCLUDING UNILATERAL RENAL AGENESIA[1]

FURTHER DATA IN SUPPORT OF THEIR INHERITANCE

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In the previous descriptive report of renal and genital abnormalities in rats (Hain & Robertson, 1936) it was suggested that the occurrence of such anomalies within a related group indicated that the defect might not occur at random, but be transmitted as an inherited tendency. As the number of defectives observed was too small to justify a conclusion, it was decided to test this hypothesis; brother-sister matings have therefore been made within the affected group throughout successive generations, and at the same time an adequate number of rats outside the group has been retained as a control. All rats born in the colony, whether related to the affected or not, have been systematically palpated bilaterally for any abnormal renal development and their external genitalia have been examined, and all stillborn young have been dissected. In this way a further series of rats possessing renal and/or genital anomalies has been obtained, and, in every instance, these have been found to be closely related to the defective rats previously described, whereas the remainder of the stock is free from the defect. The regularity with which urogenital anomalies occurred in the descendants of VII. 19 (i.e. female D—Fig. 4 in the previous paper) is well shown in the pedigree chart between pp. 100 and 101; this female possessed only one kidney and was responsible for twenty rats with various renal defects, of which three exhibited her own form of abnormality.

DESCRIPTION OF THE ABNORMALITIES

The new series consists of forty-five rats and these will be referred to by the numbers within the circles (females) and within the squares (males) on the pedigree chart between pp. 100 and 101. The Roman numeral denotes the generation to which they belong, four earlier generations appearing on the previous chart; renal defects are indicated by the kidney sign, and microphthalmia by solid circles and squares. The abnormalities were of four main types, and with hardly an exception were confined to the *left* side of the

[1] The expenses of this investigation were largely defrayed by grants (to A. M. H.) from the Medical Research Council and the Carnegie Trust for the Universities of Scotland.

body: (*a*) renal agenesia, (*b*) renal hypoplasia, (*c*) hydronephrosis, (*d*) genital anomaly without an associated renal defect.

The schematic drawings used in the earlier report are again employed for the conditions (*a*) to (*c*) but illustrate (*b*) in the female only, i.e. Fig. 3. A drawing reproduces the condition typical of renal agenesia in the male rat and forms the basis for the anatomical data given for this sex (Fig. 2). Any departures from the illustrations are described in the text. The right side of the body of all the rats was normal, unless expressly stated to be otherwise.

A. *Renal agenesia.* Seven examples of this defect were observed: females XII. 26, XIII. 5 and 6, and males XI. 15 and 20, XII. 13 and XIII. 8, of which XIII. 6 was stillborn or died soon after birth.

Fig. 1. Renal agenesia in female rat.

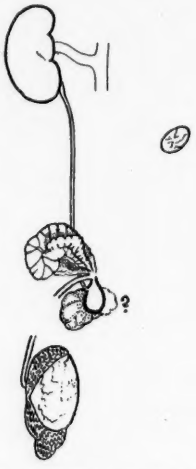


Fig. 2. Renal agenesia in male rat.

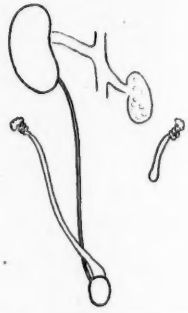


Fig. 3. Renal hypoplasia in female rat.

The typical condition is that shown in Figs. 1 (female) and 2 (male) and consists of absence of the left kidney, renal vessel and ureter. In the females there was no uterine horn on the same side but the ovary was present; in two instances in this series, however—XII. 26 and XIII. 6—the ovary also was lacking. A certain amount of hypertrophy had occurred in the right kidney of female XII. 26 which was killed when 5 months old; this kidney weighed 1·0 g., cf. 0·75 g. which is the normal for a female of the same body weight (Donaldson, 1915). In males with renal agenesia the testis was under-developed, placed high in the abdomen and lacked epididymis, globus major and vas deferens. The four males belonging to this group exhibited the condition shown in Fig. 2, but the seminal vesicle and prostate on the left side were diminutive. In XI. 15 and XII. 13 the prostate appeared to be of normal dimensions and in XII. 13 there was no seminal vesicle on the left side.

B. *Renal hypoplasia.* The condition in the female rat is illustrated in Fig. 3. Generally the hypoplastic left kidney was only one-third or one-fourth of the size of the kidney on the right side and showed cystic degeneration. As there was considerable variation both in the associated genital defects and in the extent to which ureteral development existed, the rats belonging to this section have been arranged in two groups: (i) those in which a ureter existed either in whole or in part, and (ii) those in which there was no ureter.

(i) *Partly developed ureter.* Four females and one male exhibited this form: females XI. 22 and 24, XII. 17, XIII. 11 and male XIV. 6. The kidney of female XII. 17 retained the foetal lobulation; it would seem that renal secretion had not been established in this rat as the maldeveloped ureter was not distended. This was thick-walled and connected with the cervico-vaginal junction and not with the bladder; the uterus on the same side ended in a fibrous band attached to the cervix and showed cystic dilatation.

In female XI. 24 the hypoplastic, cystic condition of the left kidney was associated with a ureter only 5 mm. long. Apparently some secretion had taken place as the ureter was a distended sac; the renal vessel was very small, however, although a normal ureteral vessel was present. Below the ovary on the same side was a portion of uterine horn only 2–3 mm. long, ending blindly.

Renal hypoplasia and cystic degeneration in females XI. 22 and XIII. 11 were accompanied by a hydroureter which was connected with the bladder but had no entrance into it. It is possible that XIII. 11 would, at a later date, have developed the condition depicted in Fig. 5 but she was killed when only 15 days old. The ovaries and uterus were normal in both animals. Comparison with a litter-mate of XI. 22 showed that there was no hypertrophy of the right kidney.

Male XIV. 6—one of four renal defectives in the same litter—had a hypoplastic kidney on the left side showing cystic degeneration of the cortex. There was partial hydronephrosis and both the pelvis and ureter were distended with fluid. The blind ureter, which was only 6 mm. long, was connected at its lower, distended end by means of the vas deferens with the globus major of the testis which was placed high in the abdominal cavity. A condition somewhat similar but less marked was illustrated in Fig. 10 of the previous report and is shown in Fig. 4*a*. Fig. 4*b* depicts a modified form encountered in four other males in this series: XI. 5, 14 and 26 and XII. 12. The seminal vesicle on the defective side in XIV. 6 was very small, but the prostate was enlarged.

(ii) *Without ureter.* Five males and two females belong to this group—males X. 25, XI. 14, XII. 4, 11 and 12, and females XII. 20 and 21. The developmental defect was most marked in XII. 11 which had neither testis nor vas deferens on the left side. The kidney measured only 5×2 mm. (cf. 12×8 mm. on the right side) and passing to it was a long fatty pedicle in which the renal vessel was twisted on itself five times. The prostate was normal on both sides but the left seminal vesicle was smaller than that on the right. In spite of a more marked degree of hypoplasia in the kidney of XII. 4—

this measured only 3 × 2 mm., cf. 12 × 8 mm. on the right side—the testis was present, although no vas deferens existed; the left seminal vesicle was very small but the prostate showed normal development. As was to be expected, the renal vessel was much smaller than that to the healthy kidney.

Rat X. 25, which was killed when 10 months old, presented a similar picture to XII. 4, but although the prostate was normally developed there was no seminal vesicle on the defective side. The left kidney was represented by a yellowish body 7·5 × 5 mm. and weighing 0·2 g., whereas the right kidney was 24 mm. long and weighed 2·5 g. Hypertrophy had occurred as the healthy kidney of a rat of the same body weight (326 g.) is 1·4 g. (Donaldson, 1915).

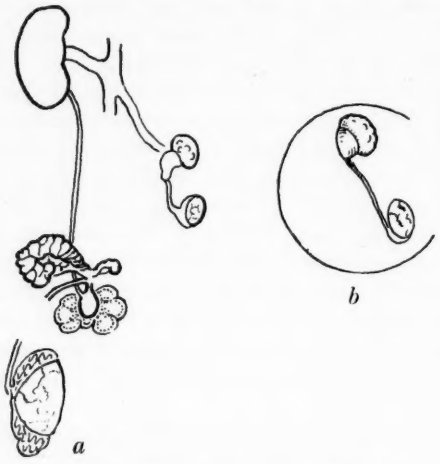


Fig. 4. Male rat with perverted vas. *a*, as in XIV. 6; *b*, as in XI. 5, 14 and 26 and XII. 12.

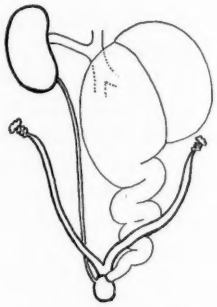


Fig. 5. Hydronephrosis in female rat.

The condition shown in Fig. 4*b* existed in male XI. 14; the kidney proper, which was highly cystic, measured 8 × 5 mm. (cf. 11·5 × 8·5 mm. on the right side and the same kidney in a litter-mate). The pelvis was greatly distended with fluid and translucent and was connected to the epididymis and globus major by means of the vas deferens which, however, was not patent at this point. The left testis was normal in size, but the left seminal vesicle and prostate were tiny; the absence of a vas at this site was confirmed and it was observed that that on the right side was normal. The renal blood supply was deficient; a small blood vessel passed over the external surface of the kidney to the vas deferens, followed along its course and passed thence to the testis.

Male rat XII. 12 presented similar features—the hypoplastic kidney on the left side measured only 7 × 2 mm., cf. right kidney 12 × 7 mm. and that of its litter-mate—11·5 × 7 mm.; in both of the latter the kidney weighed 0·2 g.,

agreeing with Donaldson's figures. Both the ureter and the seminal vesicle on the same side were wanting although the prostate was normal. The vas deferens connected the globus major with the lower pole of the kidney and from this site a vascular band passed to the hilum of the testis. The renal vessel was normal.

The two female rats with marked hypoplasia of the left kidney and no ureter were litter-mates, viz. XII. 20 and 21, and had a sister with hydronephrosis. In one female only a trace of renal tissue (cystic) existed, measuring $2{\cdot}5 \times 2$ mm., and in this rat no uterine horn was present. Its litter-mate had a cystic kidney $4{\cdot}5 \times 2{\cdot}5$ mm. and on the same side the blind uterine horn was only 2 mm. long. The ovaries and oviducts were normal in both rats.

C. *Hydronephrosis.* In rats exhibiting this condition the place of the kidney was taken, as a rule, by a large, translucent hydronephrotic sac with, occasionally, a trace of kidney substance showing cystic degeneration in the wall of the sac. The renal pelvis was greatly enlarged and also the ureter which was much kinked on itself and had no opening into the bladder. The condition is illustrated in Fig. 5, and the way in which the ureter was obstructed at its lower end is demonstrated in the detailed drawing on p. 573, *J. Anat.* **70**, 1936. In some rats, however, the kidney, though a hollow organ, was much smaller than that on the right, in marked contrast with the huge spherical sac of fluid present in others. Rats with hydronephrotic kidneys have been divided into two groups: (i) those in which the ureter was joined to the bladder though without exit, and (ii) those in which there was an entire absence of ureter or in which a hydroureter existed but ended as a blind duct some distance from the bladder.

(i) *Ureter connected with the bladder.* Eight females and three males exhibited the renal condition as this is typified in Fig. 5—females X. 15 and 16, XI. 21, 25 and 29, XII. 15, 19 and 22 and males XI. 23, XII. 14 and 18. In two of the males—XI. 23 and XII. 18—the seminal vesicle on the defective side was larger than that on the right, whereas the prostate was smaller. In all other respects the gonads and accessories were normal in both sexes.

Examples of the *small*, hydronephrotic kidney also described are provided by female XIII. 7 and male XIV. 14. The condition was associated in the female rat with cystic degeneration in the upper pole. This rat had neither ovary nor uterus and in this respect resembled its sister, in which, however, there was no trace of kidney substance on the defective side. The association of partial renal development with an absence of the gonad and secondaries on the same side has already been described in the case of male XII. 11 (p. 85) and will be observed again later (female XIV. 9, Fig. 6). In XIV. 14 the ureter was represented by a strand of tissue communicating with the bladder but not patent. The testis lay high in the abdominal cavity, with epididymis and globus major but no vas deferens. The seminal vesicle and prostate were diminutive; the renal vessel was present.

As a general rule, the right kidney showed little, if any, enlargement as

the result of the lack of function on the defective side, and although in a large number of cases the weight or measurement of the right kidney was compared with the organ on the same side in a normal litter-mate, in only two rats in this section was any measurable difference observed; this amounted to a 20 per cent increase in the right kidney of female XII. 19 and a 25 per cent increase in that of female XI. 25. Both rats were only a month old when killed and it is possible that a more marked hypertrophy would have occurred in time (*vide* X. 25 and XII. 26). Our findings are in general agreement with those of Bagg (1926). He states that "certain animals (mice) have been found with a solitary kidney that showed no appreciable enlargement. However, compensatory hypertrophy is usually associated with solitary kidney in animals examined in the adult state." When mice with extreme hypoplasia of one kidney were killed soon after birth Bagg found no hypertrophy in the functioning organ. Doctors Rytand and Addis of Standford University Hospital, San Francisco, have taken glomerular counts of the normal kidney in several of our rats in which unilateral agenesia or hydronephrosis was present, and these showed no increase in number as compared with the same kidney in a littermate.

(ii) *No connexion with the bladder.* Considerable variation existed in the expression of the abnormality in this section, which comprises six females and two males—females X. 14 and 21, XII. 16, XIV. 7, 8 and 9, and males XI. 5 and 26. In female XII. 16 (sister of XII. 17) both the cortex and the pelvis were translucent and full of fluid and a hydroureter ended as a blind duct near the bladder but had no connexion with it, although the ureteral vessel extended to the base of the bladder.

Commencing hydronephrosis with a hydroureter was present in X. 21, a stillborn female. The ureter, which was dilated in the upper two-thirds, had a sudden constriction at the junction of the middle and lower third and, from this point to the base of the bladder, was represented by what appeared to be a solid band of tissue. The ovaries and uterus were normal in both rats.

The three female litter-mates of male XIV. 6 (p. 85) belong to this section. Two of them had the small hydronephrotic kidney previously described (7×5 mm., cf. 12×8 mm.) and in each rat no trace of a ureter existed. The abnormality was associated in the one female with the entire absence of the uterine horn on the same side, and in the other with partial development of this horn, which was only 3 mm. long. In both rats the ovaries and oviduct and also the ureterine vessel were normal.

In the third female—XIV. 9—a hydroureter passed from the distended renal sac but did not communicate with the bladder. By the use of probes and with the aid of the dissecting microscope it was found that the hydroureter ended blindly at the skin of the vaginal introitus (Fig. 6). The abnormality was somewhat similar to that encountered in XII. 17 (p. 85), but in the female here illustrated neither ovary nor uterine horn existed on the left side. This female affords the third example in this series of an absence of the

gonad and its accessories in association with partial development of the kidney on the same side of the body (see female XIII. 7 and male XII. 11, pp. 87 and 85).

The abnormalities present in the remaining female in this section—X. 14, which was killed when 4 months old—are illustrated in Fig. 7. The pelvis of the left kidney was greatly enlarged, translucent and full of water. Passing from this was a hydroureter which was coiled on itself and ended blindly and abruptly at about three-quarters of its length, without any connexion with the bladder. The left ovary and uterus were normal and also the right ovary, but the *right* uterine horn was represented by a cord, which on section proved

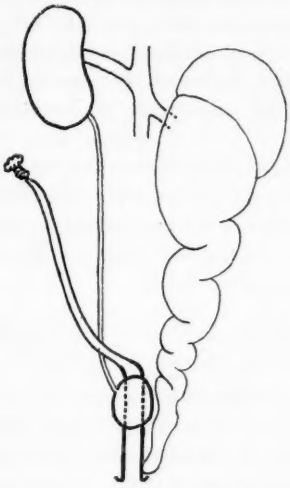


Fig. 6. Female rat XIV. 9.

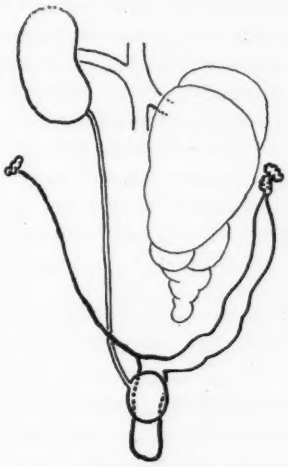


Fig. 7. Female rat X. 14.

to be solid. This was in marked distinction from the condition observed in defective rats hitherto, in all of which genital defects have occurred only on the side on which a renal abnormality existed.[1] In addition, this rat had no vaginal opening and the vagina ended in a closed sac, distended with fluid. An opening beside the anus was caused by the bursting of an abscess, the channel of which contained much granular material.

The two male rats with small hydronephrotic kidneys—XI. 5 (the left kidney measured 4×3 mm., cf. 12×8 mm. in a litter-mate) and XI. 26—resembled XIV. 6 in that there was in both a suggestion of a duct joining the kidney to the testis (Fig. 4*b*). In XI. 5 and 26, however, no ureter existed, although the main ureteral vessel was present. The small testis which was placed high in the abdominal cavity had both epididymis and globus major,

[1] Since going to press more rats with urogenital defects have been observed and in one female with hydronephrosis on the left side the *right* uterine horn was found to be a solid cord, whereas the left horn was normal.

but the vas deferens was represented only by the cephalic end which was 3 mm. long. There was neither prostate nor seminal vesicle on the defective side in either rat.

D. *Genital anomalies without associated renal abnormalities.* These were of three kinds:

(i) *Interstitial testis.* In males with this condition the testis lay between the layers of the abdominal wall, and was not descended. The epididymis was generally under-developed, and the testis was soft and smaller than that on the other side. Generally the left side was defective (IX. 24 and X. 20) but in one instance (X. 19) the abnormality occurred on the right side.

(ii) *No vaginal opening.* This abnormality was encountered in X. 14 (Fig. 7) and in rat C, i.e. VI. 19 (Fig. 3 in the previous paper on this subject), but in both rats was associated with other defects. In rat XII. 25 no other defect was present. The vagina, which had no outlet, followed the course of the rectum as far as the anus and was distended with fluid, the vaginal sac measuring 20×10 mm.

(iii) *No testis on the left side* (*adult male X.* 34). The kidneys and ureters on both sides were normal, and the seminal vesicles and prostatic glands were well-developed. On the left side, however, the vas deferens ended in a blind duct about 20 mm. long and the testicular vessel was absent. This male fathered a female with a hydronephrotic kidney, viz. XI. 29.

THE VARIOUS FORMS OF RENAL AND ASSOCIATED GENITAL DEFECTS IN RELATION TO THEIR MORPHOLOGICAL DEVELOPMENT

As in the earlier series, renal defects were confined to the left side of the body, and, with two exceptions, the associated genital anomalies were similarly restricted. Owing to the much larger number of defectives available in the new series and the wider range of abnormalities encountered, as well as the various modifications and the typical forms by which these were expressed, a fuller examination of their nature has been attempted with a view to throwing some light on the process by which the defect may have developed.

The attendant anomalies in unilateral renal agenesia are readily understood: the absence of renal vessel, ureter and uterus or vas. The development of the Müllerian and Wolffian ducts is so intimately related to that of the kidney anlagen that the factor inhibiting the development of the latter would suppress the former also. This was discussed in the earlier descriptive report. It is not surprising, therefore, that in those rats in which renal development was not totally suppressed—in cases of hypoplasia and hydronephrosis—the degree of maldevelopment of the ureter ran almost parallel with that of the uterus in the female and of the vas in the male. Thus, for example, one finds a fibrous ureter associated with an equally non-functioning strand for uterus; a ureter 5 mm. long accompanies a uterine horn of similar dimensions, and where there is no ureter—which occurred in certain examples of hypoplasia and hydronephrosis in addition to the accepted cases of renal agenesia—there is

likewise an entire absence of any uterine development on the same side, and occasionally, even, of the ovary also.

Owing to the greater complexity of the secondary sex apparatus in the male, greater variety existed in the abnormalities encountered in this sex; counterparts of conditions found in the female were observed. In one, the ureter is represented by a solid strand and there is no vas deferens; in another, marked renal hypoplasia and an entire lack of ureteral development are associated with an absence of the vas, and (in rat XII. 11) also of the testis. Development of the prostate and seminal vesicles varied in rats exhibiting the same degree and form of renal defect (in such rats it was observed, moreover, that the prostate apparently develops independently of the seminal vesicle). Although renal deficiencies were accompanied also by defects in the vas deferens, actually only three out of seven males in which there was an entire absence of ureter showed no trace of a vas. The remaining four, together with a fifth rat in which the ureter was partially developed but ended in a blind duct, were peculiar in that, in each case, the vas connected the globus major with the kidney (or, in the last named rat—XIV. 6—with the blind end of the distended ureter); also, in two of the rats, the course of the connecting vas was traversed by a blood vessel linking kidney and testis. It is intended to examine, in some detail, the possible morphological development of this peculiarity, not only in view of its interest, but because of the light that it is hoped may be thrown on the origin of other urogenital anomalies herein described. The authors are indebted to Dr Peter Gray of the Zoology Department, Edinburgh University, for the suggested explanation and for the schematic drawings appended.

Fig. 8 illustrates renal development in the theoretical normal. From the pronephros there passes the archinephric duct which, in the course of its development, makes contact with the urogenital sinus. Round about it the mesonephros develops, and a connexion between the latter and the testis is established and persists, since the upper end of the mesonephros becomes the epididymis. From the archinephric duct near its lower end, i.e. at the caudal end of the mesonephros, there springs the bulbous epithelial bud which, by outgrowth, develops into the ureter and pelvis of the kidney. This bud is covered by a cap of nephrogenic tissue derived from the lower extremity of the mesonephric blastema which goes to form the kidney substances.

This revision of facts gives a clearer understanding of Figs. 9 and 10 which illustrate what has possibly happened in the males with a perverted vas. In Fig. 9 a less extreme condition is typified than in Fig. 10 and may be taken to represent rat XIV. 6 in which a portion of ureter existed. In both illustrations the archinephric duct is shown as failing to complete its connexion with the anlage of the bladder, and, in consequence, both the mesonephros and the ureter are shortened. In Fig. 9*b* the condition as found in the adult is shown; the archinephric duct (vas deferens), instead of extending to the bladder, is joined to the lower end of the shortened ureter; the kidney, which arose from

the incompletely developed mesonephros, is, in turn, hypoplastic or hydronephrotic. The more extreme condition exhibited by four males is typified in Fig. 10*a* and *b* from which it appears that the so-called vas in these rats is really a fusion of the vas and ureter, though not patent at the renal end and not connecting with the hilum of the kidney.

It would seem that the adverse factor which suppressed renal development in rats with agenesia operated at a slightly later date in those animals in which hypoplasia was associated with an absence both of ureteral development and of vas; also, that the five rats with "perverted vas" possibly occupy a position intermediate between the latter and those females in which a hydronephrotic

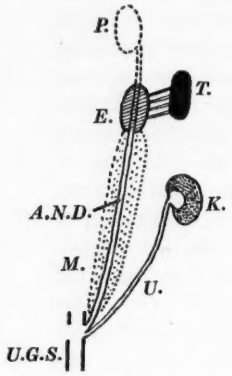


Fig. 8.

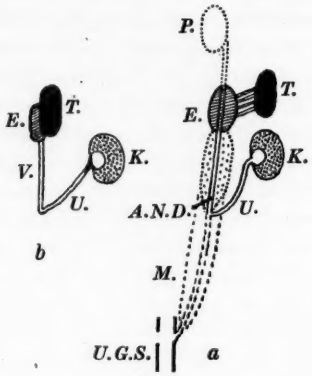


Fig. 9.

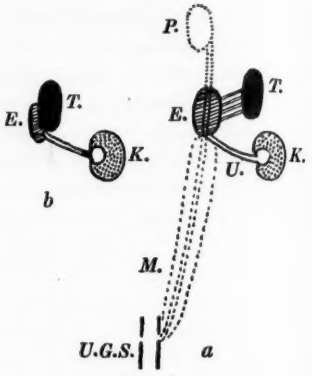


Fig. 10.

Fig. 8. Schematic diagram showing renal development in the theoretical normal. *P.* pronephros; *E*, epididymis; *T.* testis; *A.N.D.* archinephric duct; *M.* mesonephros; *K.* kidney; *U.* ureter; *U.G.S.* urogenital sinus; *V.* vas deferens.

Fig. 9. Schematic diagram showing possible course of renal retardation as this occurred in Male XIV. 6 (i.e. Fig. 4 *a*). *a*, developmentally; *b*, in the living rat. Lettering as for Fig. 8.

Fig. 10. Schematic diagram showing retarded development of mesonephros and ureter which may have given rise to condition in Fig. 4 *b*. *a*, developmentally; *b*, in the living rat. Lettering as for Fig. 8.

kidney was associated with a hydroureter having a stricture at its entrance into the bladder. With a single exception (female XIII. 7) the uterus and ovary on the defective side in such rats showed normal development.

The occurrence of genital anomalies independently of renal defects, together with the absence of a gonad in rats in which the kidney was either lacking or only partially formed, may be explained only on the assumption that, owing to the development of the gonad at a site on the urogenital ridge adjacent to and in contact with the early nephric rudiments, the factor which caused abnormal development of the latter might, either in addition, or alternatively, cause malformation of the former. The irregularity sometimes encountered in the attendant anomalies may, perhaps, be accounted for in this way.

Two female rats exhibited special features requiring comment: XIV. 9 and X. 14. In the former (Fig. 6) a condition found in early embryonic development persisted, viz. the ureter maintained the position which it had in the cloaca before it acquired a separate opening, and, by occupying a more cephalic position, communicated with the bladder. The lack of an outlet was responsible for the hydronephrosis present in this female. The complicated picture presented by female X. 14 (Fig. 7) suggests that in this rat there occurred a developmental arrest of a more general and diffuse character than that elsewhere encountered. The fact that rats within the related "renal" group which gave promise of large litters frequently produced only two or three youngsters, having absorbed the others or eaten them at birth, suggests that the latter may not have been viable owing to the number and complicated character of the defects exhibited. Beaver (1933) cites such a case in man, and Bagg (1926, 1929) found many such in mice.

The occurrence of eye defects, particularly microphthalmia, in the same stock has already received comment (Hain & Robertson, 1936). The association between the eye and the kidney is obscure; according to Bagg (1924), who experienced a similar association in a strain of mice descended from X-rayed animals, "an experimental disturbance during the very early embryonic period is likely to result in various eye defects, whereas an identical disturbance acting somewhat later will result in defective brain or branchial systems, and still later in malformation of the viscera". In this connexion the two litters produced by XIII. 1 and 2 are of peculiar interest: the first contained five rats with eye defects, the second had four with renal defects. This association between eye and kidney abnormalities is referred to again later.

It is possible to account for the abnormalities described only on the basis of developmental arrest; the nature of the factor causing the arrest in development is not clear. Under-nutrition of the embryos is exceedingly unlikely as such abnormalities would then be of frequent occurrence in the litters carried by suckling females, which has been demonstrated not to be the case (Hain, 1934*b*).

That faulty diet and lack of exercise can have serious effects on the development and function of the kidney is well known. Spontaneous nephritis in rats fed on a diet deficient in vitamin A is recorded by Jackson (1924) and recalls the term "renal rickets" in man. The rats employed in this study have been fed on a standard diet for the past 6 years, and this is so well balanced in vitamin content that it is unlikely that any dietary defect is responsible for the abnormalities. Flaked maize, cod-liver oil, liver and yeast powder, milk, green vegetables and a little salt are given daily, and, in all material points, the quality of the stock bears comparison with the original Wistar rats from which it sprang (Hain, 1934*a*).

In a histological study of the embryos of a large number of mice of Bagg's X-rayed anomalous strain, Brown (1931) investigated the retardation processes at different stages of embryological development. She attributes the condition

to an aberrant vascular system in the embryo resulting in deficient nutrition, whereby, when the stress of twisting of the posterior region of the early embryonic body occurred, the deficient excretory and drainage mechanisms failed to unite. Abnormally large iliac and umbilical veins were found in embryos of fourteenth-day development and undue pressure from these, together, perhaps, with pressure from the foetal membranes in twisting, played an important part in effecting those strictures which arrested the development both of the ureter and of the metanephros. Definite retardation points were observed in the ureter anlagen and the nature and extent of renal deficiency were related to the stage at which ureteral growth was arrested. She offers no satisfactory explanation for the association of eye and kidney defects repeatedly found in the same mice.

UROGENITAL ANOMALIES AS INHERITED DEFECTS

The pedigree chart reproduced between pp. 100 and 101 shows the descent of sixty rats with urogenital abnormalities, of which fifteen rats appeared on the previous chart but were necessarily included here both for purposes of relationship and to illustrate the incidence of the defect in the different generations. Only those rats are shown on the chart which were directly involved in the inheritance of urogenital anomalies; an exception has been made, however, where it was possible to show also the relationship to rats with microphthalmia and other eye defects. It will be noted that the pedigree table consists of six groups of rats, all of which are descended from a single litter in the first generation shown on the original chart. In Table I the six groups are referred to by the chart number of the female heading each group in generation V, and the incidence of both urogenital anomalies and eye defects is shown not only in each generation but also in each group. The number of rats comprising the nine generations reviewed, viz. 5007, does not represent the total number of living offspring produced, as, owing to the exigencies of space, and the needs of other scientific workers, rats were disposed of, which, had they been retained for breeding purposes, might have swelled the ranks of the defectives. In addition, considerable numbers of rats related to defectives were used in experiments involving abortion or dead litters (this accounted for the fading out of groups II and VI) or necessitating the deaths of the rats employed; and finally, prior to the ninth generation the presence of renal defects was ascertained in the female only by laparotomy or post-mortem examinations and it was obviously impossible to examine the whole stock in this way. From the ninth generation onwards it was found possible, by means of palpation, to detect any renal defect before the fourteenth day of life; as a result, in this series, the majority of the rats described were killed when only 3–5 weeks old. Although the above facts detract from the value of the report as a genetic study, it is unlikely that they affect materially the percentage of incidence of the defect.

Table I. *Showing the occurrence of urogenital anomalies and eye defects in ten generations of rats and descent from six females in the first generation*

Generation	No. of living descendants of						No. with urogenital defects: descendants of						No. with eye defects: descendants of					Total alive	Total with urogenital defects	Total with eye defects	No. of ♀♀ that gave birth to total in each generation
	V. 3	V. 4	V. 8	V. 10	V. 13	V. 15	V. 3	V. 4	V. 8	V. 10	V. 13	V. 15	V. 3	V. 4	V. 8	V. 10	V. 13				
VI	6	23	7	12	6	19	—	—	—	—	—	1♀	1♂ 1♀ 1♂ hydro.*	—	1♂ 2♀♀	—	1♀	73	1	6	6
VII	37	150	77	65	65	31	—	2♂♂	—	1♂ 1♀	—	2♂♂	2♂♂ hydro.*	1♂ 1♀	—	1♂ 2♀♀	1♂ hydro.*	425	6	5	27
VIII	149	67	140	108	100	48	—	—	1♂	— 2♀♀	—	—	3♂♂	—	1♂	3♂♂ 2♀♀	1♀	612	3	10	46
IX	90	59	52	207	122	—	—	—	—	1♂ 1♀	—	—	7♂♂ 6♀♀	—	3♂♂ 3♀♀	2♂♂ 1♀	—	530	2	22	50
X	134	50	110	334	193	—	—	—	3♂♂	4♂♂ 4♀♀	1♀	—	2♂♂ 1♀	—	—	1♂ 3♀♀	—	821	12	7	63
XI	110	126	148	519	250	—	—	—	1♂	5♂♂ 5♀♀	—	—	5♂♂ 2♀♀	—	—	—	—	1153	11	7	112
XII	86	86	122	380	304	—	—	—	1♂	5♂♂ 7♀♀	2♀♀	—	—	—	—	2♂♂ 1♀	4♂♂ 4♀♀	978	15	11	114
XIII	68	26	100	15†	131	—	—	—	1♂ 4♀♀	—	—	—	—	—	—	—	—	340	5	—	35
XIV	65	—	6	—	4	—	1♂ 3♀♀	—	1♂	—	—	—	5♂♂ 6♀♀	—	—	—	—	75	5	11	13
Totals	745	587	762	1640	1175	98	1♂ 3♀♀	2♂♂	8♂♂ 4♀♀	16♂♂ 20♀♀	3♀♀	2♂♂ 1♀	23♂♂ 16♀♀ 3♂♂ hydro.*	1♂ 1♀	5♂♂ 5♀♀	9♂♂ 9♀♀	4♂♂ 6♀♀ 1♂ hydro.*	5007	60	79	466
							Totals 4	2	12	36	3	3	39	2	10	18	10				

* hydro. = hydrocephalic. † Others too young to mate.

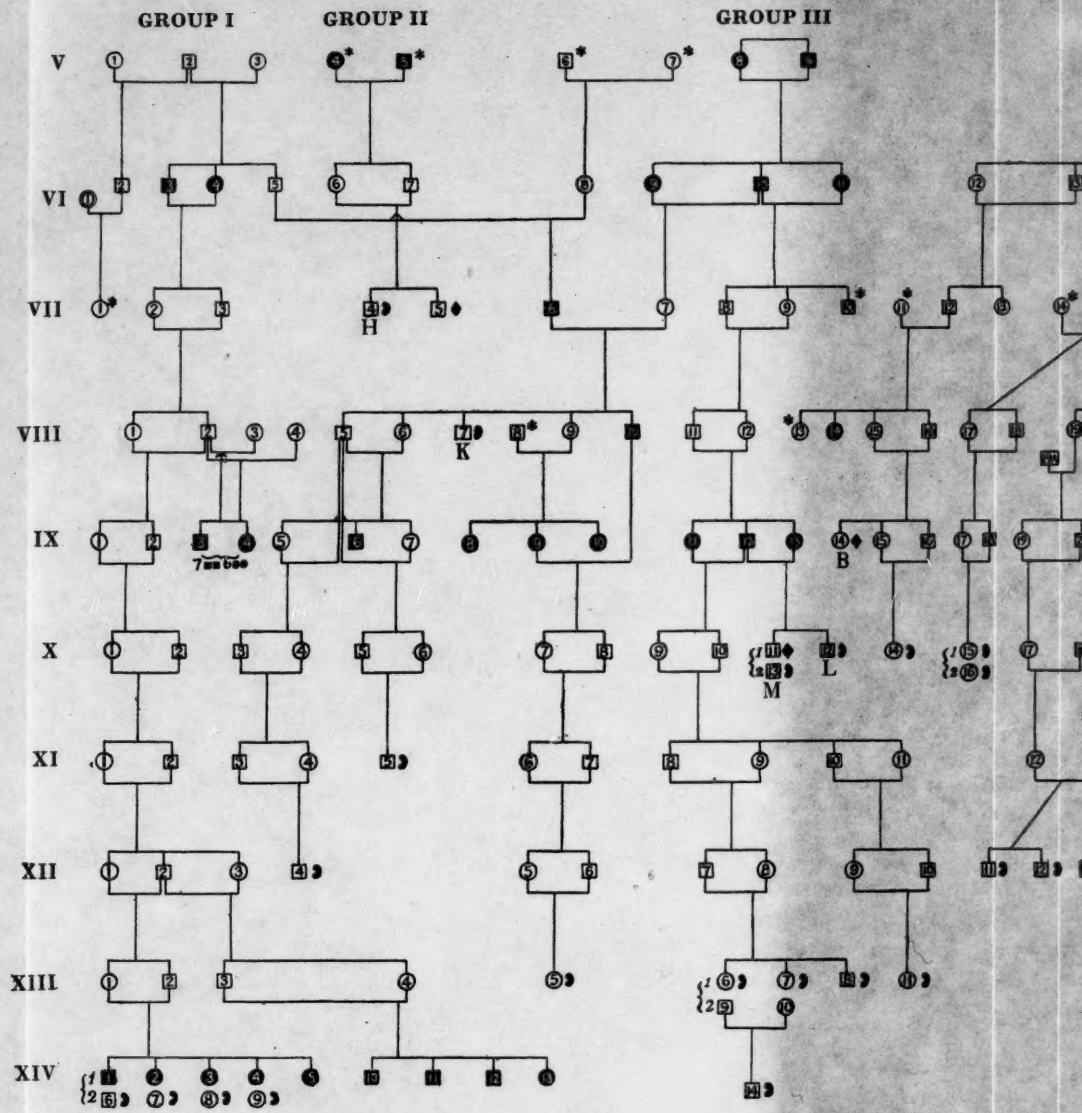
If the chart and Tables I and II be examined it will be seen that, of 466 rats that gave birth to live litters, thirty-nine produced a total of sixty urogenital defectives. It is striking that 60 per cent (or thirty-six) of these occurred in Group IV, and were descended from female V. 10, and that $33\frac{1}{3}$ per cent of the total (i.e. twenty rats) are traced back to a single rat—VII. 19. In proportion to the number of rats born, rat V. 8 gave almost as high an incidence, but whereas in this case the defect was spread over seven generations of rats, in VII. 19's the abnormality was confined—with a single exception and excluding VII. 19 herself—to two generations.

The percentage of urogenital defectives within the whole related stock indicates a high incidence, viz. 1·2 per cent or approximately 1 in 83; renal agenesia, however, is apparently as rare as in man for only seven examples have been encountered, i.e. 1 in 715 rats. The data collected from the literature by Collins (1932) showed that renal agenesia was observed in man in 1 in 920 necropsies; his own figures placed the incidence higher, viz. 1 in 705, and Campbell (1928) has recently observed 1 in 400 (personal communication). If the incidence of urogenital anomalies within a restricted group such as that of female VII. 19 be contrasted with that in related animals as a whole, the increase will be found to be sufficiently significant to warrant the defect being considered inherited. Among 480 descendants of rat VII. 19, comprising generations XI and XII, there occurred twenty rats with urogenital anomalies, i.e. over 4 per cent were defective. The high familial incidence is plainly obvious on the chart; Table II demonstrates the inherited character of the abnormalities in other ways, most notable of which is the occurrence of animals with renal and/or genital defects in successive litters born to the same parents, and the frequency with which the mating of normal litter-mates of such defectives again produced abnormals. The pedigree chart shows several instances. It will be observed that the percentage of urogenital defectives rises from 1·2 per cent in the related stock to almost 21 per cent in affected litters or 9 per cent in all the offspring born to parents of affected animals.

Lest it be suggested that an unhealthy condition either of the ovum or of the uterus was responsible for the maldevelopment of the embryo in its early stages, figures have been given in Table II showing the number of normal litter-mates both within affected litters and born in previous or subsequent litters—for frequently two and three normal litters were born to parents of affected offspring. Apart from the consideration of litter size, the general physical fitness both of the defective rats themselves and of their parents renders the hypothesis unlikely, although it does not preclude the possible existence of some adverse influence affecting the ovum at an early stage of segmentation and thus providing a physiological as well as a genetic basis for the anomaly, such as was put forward by one of us (Hain, 1936) in the case of microphthalmia in a recent study of the inheritance of eye defects in the same stock. This is discussed more fully below.

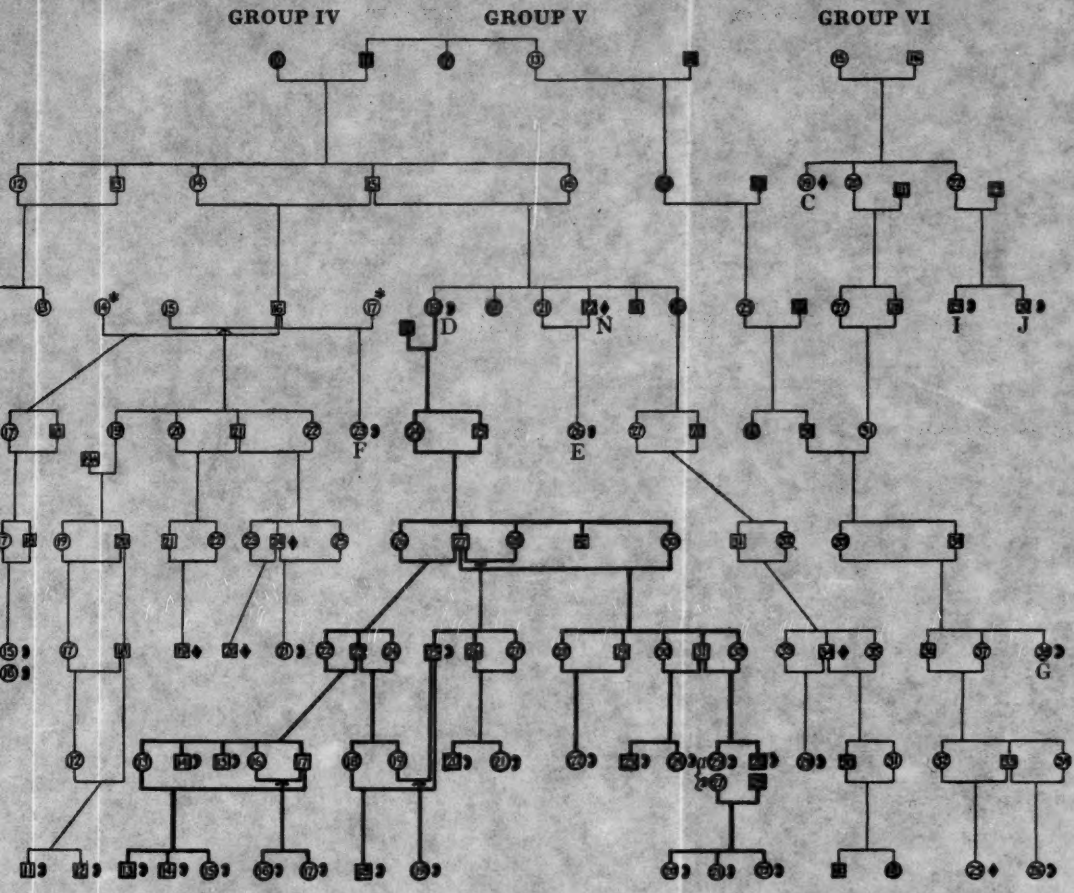
In the six groups of rats covered by this study, eye defects were hardly more

Pedigree Chart of rats having rena



* { V. 4, 5, 6 and 7 cousins;
VII. 1 same as VII. 17;
VII. 10 same as VIII. 8;
VII. 11 in direct descent from V. 11 × 12;
VII. 14 same as VIII. 13.

aving renal and genital anomalies



○ = female; □ = male;
◎ = female outcross; ▣ = male outcross (i.e. from a different strain of rats);
●■ = microphthalmics; ♦ = genital abnormality only;
❩ = kidney defects.

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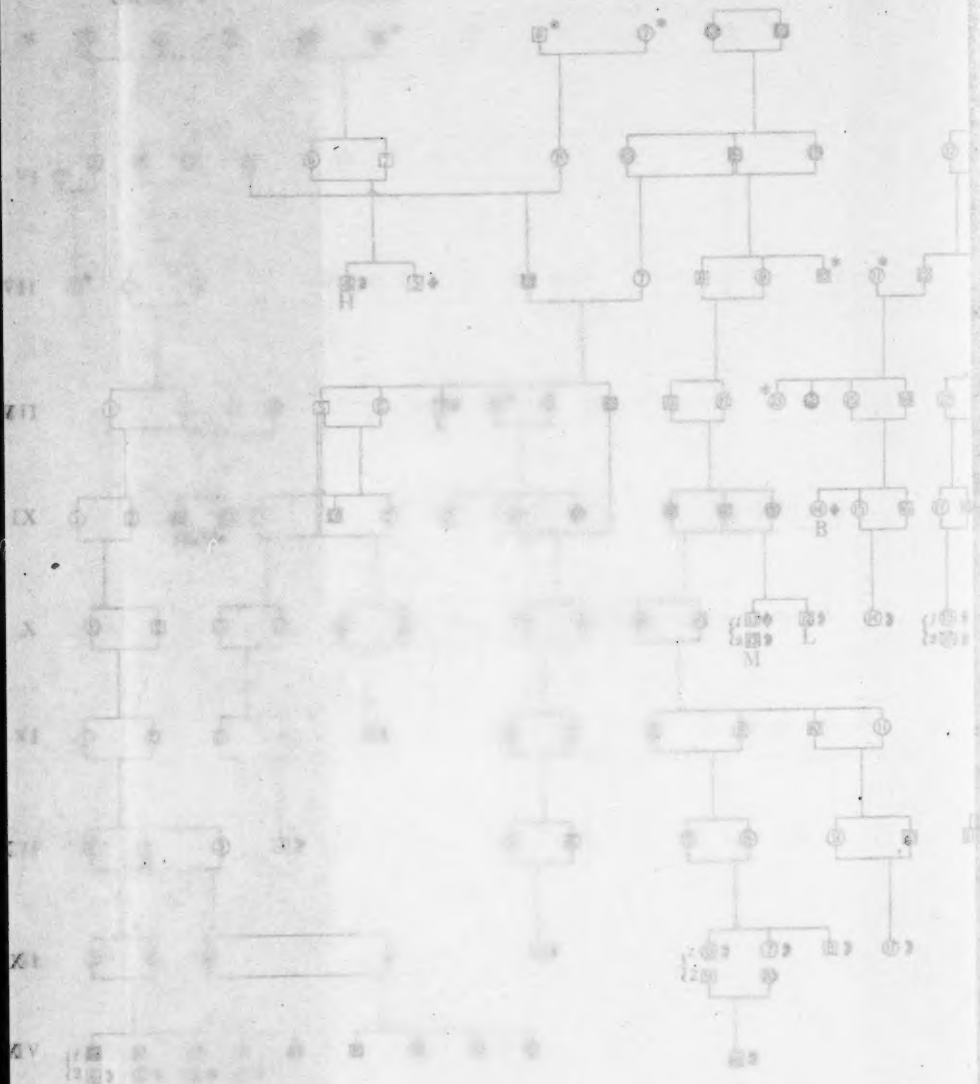
Pedigree Chart of rats hav

GROUP III



V. 4, 5, 6 and
VII. 1 same as
VII. 10 same as
VII. 11 in direct
VII. 14 same as

Table II. *Showing the proportion of defectives to normals born to the same female*

Generation of rats	Mothers of defective rats	No. defective ♂♂	No. defective ♀♀	No. normal in same litter	Total normal to same ♀
VI	V. 15	—	1	8	18
VII	VI. 6	2	—	9	19
	VI. 16	1	—	5	24
		—	1	10	
	VI. 22	2	—	6	17
VIII	VII. 7	1	—	10	33
	VII. 17	—	1	11	33
	VII. 21	—	1	3	7
IX	VIII. 15	—	1	5	5
	VIII. 22	1	—	5	5
X	IX. 33	—	1	6	47
	IX. 32	1	—	5	5
	IX. 28	1	—	7	7
	IX. 25	—	1	8	10
	IX. 23	1	—	11	18
	IX. 22	1	—	6	24
	IX. 17	—	1	5	31
		—	1	10	
	IX. 15	—	1	4	15
	IX. 13	2	—	2	21
		1	—	9	
XI	X. 27	1	1	8	29
	X. 28	—	1	10	16
	X. 30	1	1	5	23
	X. 32	1	1	10	25
	X. 22	1	—	4	24
		1	—	9	
	X. 33	—	1	4	11
	X. 6	1	—	5	15
XII	XI. 4	1	—	9	18
	XI. 12	1	—	3	11
		1	—	3	
	XI. 13	2	1	4	16
	XI. 16	—	2	5	13
	XI. 18	1	—	10	10
	XI. 19	—	1	2	2
	XI. 27	—	3	5	15
	XI. 32	—	1	10	10
	XI. 34	—	1	7	24
XIII	XII. 5	—	1	6	12
	XII. 8	1	2	11	24
	XII. 9	—	1	4	10
XIV	XIII. 1	1	3	6	13
	XIII. 10	1	—	2	2
Totals	39 ♀♀	29	31	287	662
		60			

Percentages of urogenital defectives:

(*a*) To normals in same litter (287) ... ... 20·9% or *c.* 1 in 5.
(*b*) To normals to same ♀ parent (662) ... ... 9·0% or *c.* 1 in 11.
(*c*) To all related stock (5007) ... ... 1·2% or *c.* 1 in 83.

prevalent than defects of the urogenital system, which, owing to the difficulty of detection, were apt to be missed. Collins (1932) included microphthalmia in his list of anomalies found associated with renal agenesia in the literature bearing on the human subject. However, a comparison of the numbers with urogenital and eye defects totalled for each group in Table I does not show any association between the frequency of the two abnormalities in any group. Of special interest are those occasions on which rats having eye defects and others with urogenital defects were born either in the same or in successive litters to the same parents, e.g. VII. 19–24, VIII. 7–10, X. 11–13 born to blind parents, XIV. 1–9 and XII. 23, 24 blind where one might reasonably have expected urogenital abnormalities. The most interesting case is that of rat XIII. 1, which, after having a litter containing five rats with microphthalmia, produced four with urogenital defects.

The occurrence of eye and kidney defects in the same stock of rodents necessitates a discussion of the results obtained by Bagg in rats (1922) and more notably in mice. Developmental arrests which were noted in the surviving young of pregnant rats irradiated on the nineteenth day of gestation, consisted especially of brain defects and also of cataract and gross eye abnormalities. Blood vascular disturbances were noted also in the young of rats irradiated several days before mating. The experiments were repeated in a modified form on mice (Bagg & Little, 1924; Bagg, 1926, 1929) and demonstrated that abnormalities can be induced in the descendants of irradiated mice, without any immediate physiological effects being manifested in the irradiated animals themselves or in their litters. The first eye defect occurred in the third generation removed from the treated animals, and kidney abnormalities developed four generations later (Bagg, 1926). Eye and kidney defects were frequently observed in the same individuals, and the mortality was high owing to the frequency with which both kidneys were missing. Club-feet and skull asymmetry also appeared with relative frequency and were associated with the defects described. In the opinion of Bagg and also of Bagg & Little the tendency to abnormality in structure was associated with blood extravasations in the developing young of strains showing a high percentage of defectiveness. These caused lesions which interrupted the normal rate of development of definite parts of the embryo, the wide range of structural abnormality being accounted for by the different periods of prenatal development at which the vascular disturbances occurred and by their severity.

These facts have been given at some length not only because similar eye and kidney defects have occurred in the stock of rats used by us, but the interval of time which elapsed between the first appearance of each of the two defects was the same as was observed in Bagg's mice, viz. four generations. There is, however, no association of irradiation with the ancestors of any of this stock, or with any member of the stock itself. Moreover, microphthalmia first appeared in the original American stock of Wistar rats (of which the Edinburgh stock is a branch) in 1913, and a study of its inheritance and of other

eye defects was made by Dr Helen Dean King (1931). No other structural anomalies were found in association with the eye defects, but Dr Hammett of the Wistar Institute is reported by Bagg (1926) as having found a single adult female rat with a solitary kidney, "which was the only animal found with this condition in a group of several thousand autopsies".

The similarity between the expression of the developmental arrests described in our rats and Bagg's mice is sufficiently striking to admit the possibility of the same causal agency, the outward expression of the arrest being dependent on the "critical period" (Stockard, 1920) at which this operated. Certain features prominent in Bagg's mice do not exist in our rats:

(*a*) Whereas almost invariably two or more defects existed in the same mouse, only one—either eye *or* kidney—was found in our rats.

(*b*) By inbreeding, the incidence of the defects was immeasurably increased in mice (Bagg, 1926). An increase was also observed in the rats, but this was by no means commensurate with Bagg's figures. According to him, the characters appeared as recessives following Mendelian laws, and no abnormal-eyed animals were found in the first generation of an abnormal by normal outcross. This was strictly contrary to the findings of Hain (1937) as is shown by two examples on the pedigree chart between pp. 100 and 101, viz. rats VI. 17 and VIII. 29.

(*c*) It is remarkable that in none of Bagg's defective mice were renal defects associated with genital defects on the same side of the body such as have been a prominent feature in the rats herein described.

(*d*) Whereas the renal defects in rats were confined to the left side, in mice they were almost evenly distributed, though with a tendency to occur more often on the right side; however, a marked tendency for clubbing of limbs to occur on the left side of the body is recorded by Bagg (1929).

From a study of the facts presented by the pedigree chart and Tables I and II it is concluded that, although the evidence indicates that renal anomalies are inherited characters, their mode of inheritance, as in the case of microphthalmia, is not a straightforward one. The data are consistent with the existence of multiple factors or of a dominant factor with poor expression, the manifestation of which is determined both by genetic and physiological factors. A comparison of the charts for microphthalmia (Hain, 1937) and urogenital anomalies indicates a more straightforward mode of inheritance for the latter than for the former.

SUMMARY

1. As the result of the inbreeding of rats related to animals showing urogenital defects, the incidence of the latter has been increased. Additional forms of the anomaly have been obtained and are described.

2. An attempt is made to relate the nature and degree of renal and genital anomaly to the conjectured arrest of renal development in early embryonic life.

3. The abnormalities are inherited and are probably associated with microphthalmia although they have not appeared in rats having eye defects. It is probable that both anomalies are due to arrests in the development of the embryo, the outward manifestation being determined by the stage at which the disturbance occurred.

4. The similarity between effects produced by Bagg as the result of irradiating mice and those that have been found in the rats here described is discussed.

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VARIATIONS FROM NORMAL GAIT AFTER MUSCLE SECTION IN RABBITS

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In this paper an account is given of an investigation which was made to determine what changes could be observed in the gait of an animal after the throwing out of action of one or more of a group of muscles acting on a joint. For this purpose, the rabbit was selected, and the muscles which were chosen upon which to perform the operations were the superficial and deep muscles of the calf. These include all the muscles which are responsible for the flexion of the ankle joint, and, except the small intrinsic muscles of the foot, they are the only ones which flex the toes and the joints of the foot. It was felt that by eliminating these one after the other it would be possible to determine the alterations in the ability of the animal to flex its ankle and the joints of the foot and toes.

The muscles of this group in the rabbit vary in some important details from the arrangement seen in the human, and this must be borne in mind when the results of the experiments are considered. In a series of twenty rabbits, the outer head of gastrocnemius weighed on the average 2·35 g. and the inner head of gastrocnemius 1·59 g. Their origin and insertions were similar to those found in man. The plantaris is relatively a large muscle and weighed 1·62 g. on the average. Its tendon of insertion winds round that of the gastrocnemius and passes over the heel to run into the foot. As it passes over the heel it gives off two lateral expansions which are inserted into the sides of the calcaneal process. The tendon, upon arrival in the foot, broadens out and sends slips to each of the individual toes. These slips are perforated by the corresponding tendons of the deep flexor muscles. The soleus, on the other hand, is relatively small, weighing on an average 0·55 g. It arises only from the head of the fibula, and its tendon of insertion joins with that of the gastrocnemius to be inserted into the os calcis. It is made up of "red" fibres, this differentiating it from the other muscles which consist of "white" fibres. In this investigation, the deeper muscles have been grouped together as the deep flexors and weigh on the average 1·67 g. This has been done because it was found difficult to separate them into individual muscles. This mass arises both from the tibia and the fibula, and its tendon of insertion divides into slips which after perforating the corresponding tendons of the plantaris are inserted into the distal phalanges of the toes. From the above description it will be observed that the gastrocnemius and soleus are plantar flexors of the ankle joint and the deep flexor, a flexor of the toes. The plantaris, from its arrangement, can be both a plantar flexor of the

heel and a flexor of the toes, and it is difficult to state which of these actions is the more important, but these experiments show that it is a powerful flexor of the toes and suggest that it is relatively less important as a plantar flexor of the heel. The weights given for each of these muscles are for the purpose of comparison and are not the average of full-grown individuals, as a fair number of the animals in the series from which the measurements were taken were young. The average weight for the group was 890 g.

The method which was adopted for destroying the action of the muscle was to remove a portion of its tendon of insertion. This had the advantage of destroying the individual action of the muscle while its nerve supply was preserved intact, and thus afferent impulses could still be conveyed from the muscle to the central nervous system.

A few preliminary experiments showed that dissection of the tendon of insertion of any one muscle influenced the gait to such a small extent that it was impossible to detect this by means of the naked eye. It was felt that such alterations might be more readily detected by means of slow-motion cinematography, and this was employed in the series of experiments which are described in this paper. The routine of the experiments was as follows. A record of the normal gait of the experimental animals was obtained by exposing films at the rate of 64 frames per second. As these are afterwards run through the projector at 16 frames a second, the rate at which the animal appears to move as seen on the screen is a quarter of that of the normal gait. After a record of the normal gait had been obtained, the tendons of the individual flexor muscles were divided at 2-daily intervals, and on the intervening days similar records to those of the normal gait were made with the ciné camera. As the group of muscles consisted of the outer head of gastrocnemius, the inner head of gastrocnemius, plantaris, soleus and the deep flexors, an experiment was completed within 10 days of the section of the first muscle. This was well within the time required for reformation of the tendon and return of function to the muscle which, as the author has been able to show (1), does not occur until after 3 weeks from the date of tendon section.

The order in which the tendons were cut varied throughout the series of animals, so that it was possible to study the effects of eliminating the action of different combinations of the members of the group of muscles. Complete cinematograph records were obtained at all stages of the experiments and were examined by being projected on the screen.

Investigation in which the cinematograph is used offered difficulties in publication in that it is impossible for the reader to see the actual film. To surmount this difficulty to a certain extent, the author has made use of the same method which he employed in a recent paper (1), by projecting the film on to a roll of paper and tracing the position of the limb in every consecutive frame throughout the cycle of movement. If the animal was moving very slowly, the number of frames in the cycle was very large, and to reduce this, outlines were made from every other frame in the cycle, or occasionally

every third frame. It is hoped by this means that the reader will be able to follow the description of the changes observed in the projected films, but it must be remembered that in the film, movements could be seen more clearly than in the outline drawings.

The author has also examined these movements by comparing the variations in the amplitudes of the movement of the individual joints, but as these, although more accurate, merely confirm what has been seen on the film, it is not thought necessary to add to the length of the paper by giving them here.

RABBIT 13

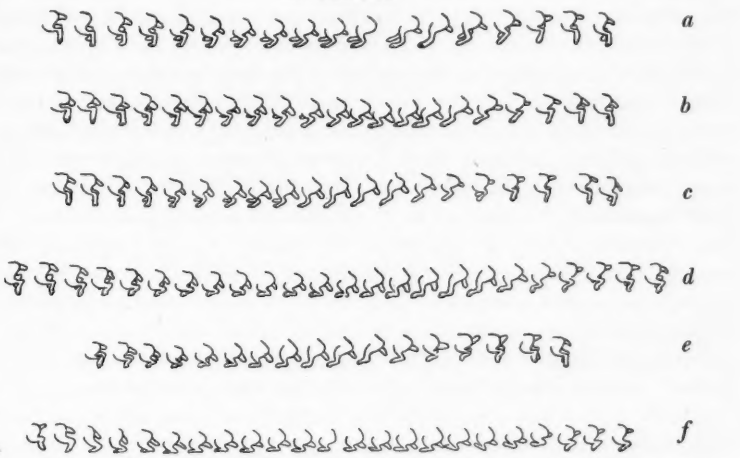

Fig. 1. Rabbit 13. *a*, Normal gait; *b*, outer head of gastrocnemius cut; *c*, both heads of gastrocnemius cut; *d*, gastrocnemius and soleus cut; *e*, gastrocnemius, soleus and deep flexors cut; *f*, all muscles cut.

In this animal, the tendons were cut in the following order:

(1) Outer head of gastrocnemius,
(2) Inner head of gastrocnemius,
(3) Soleus,
(4) Deep flexors,
(5) Plantaris.

After section of the outer head of gastrocnemius (Fig. 1*b*), no alteration in the gait of the animal could be detected. After both heads of gastrocnemius had been divided (Fig. 1*c*), the movements of the knee and the ankle still remained unchanged. There was, however, a slight difference in the position of the toes, and during the support period, while the feet were on the ground, the toes appeared to be rather less dorsiflexed than during the earlier stage of the experiment. This would suggest that the plantaris and deep flexors are now more strongly contracted. After section of the gastrocnemius and soleus, there

is a distinct increase in the amount of the dorsiflexion of the ankle during the suspense period while the feet are off the ground. This is clearly due to the anterior muscles now having less opposition to overcome. During the support period, the raising of the heel is now delayed and only occurs late in the support period just before the foot is leaving the ground. The position of the toes is clearly altered, and there is now no doubt that the plantaris and the deep flexors are kept in a state of marked contraction. Fig. 1*d* shows that the toes are flexed at all stages of the locomotory cycle, and even when the extensors are active during suspension they are unable to counteract the action of the flexor muscles. These changes which have occurred through the division of the soleus are most striking. As has been mentioned above, it is in the rabbit a relatively small muscle, yet, so long as it remains intact, the loss of locomotory power from the division of the tendons of the large gastrocnemii is negligible. This would suggest that the soleus, in at least certain stages of the cycle, must be in a state of hypercontraction and exerting a power much above that which it normally attains. Such a hypercontraction is clearly present in the two remaining muscles, the plantaris and the deep flexor, for, as has already been mentioned, the toes are in a condition of marked plantar flexion which the extensors of the toes are unable to counteract. These findings therefore suggest that when a muscle or muscles are divided, the remaining muscles, acting upon a joint, attempt to counteract this loss by greater contraction, and if the quantity of muscle which has been thrown out of action is not overwhelmingly great, they succeed in doing so. After section of the gastrocnemius, soleus and deep flexors, there is little further change in the movement of the ankle joint. The toes are slightly less flexed than in the last experiment, but this diminution in the amount of flexion is very slight when it is considered that a considerable amount of the flexor force, as represented by the deep flexor muscle, has been thrown out of action. It would suggest that once more the remaining muscle, the plantaris, is acting with still greater force. It is able to counteract the whole of the extensors of the toes, because at no stage of the cycle are the toes anything but flexed. After all the muscles have been cut (Fig. 1*f*), the foot is carried forward during the suspense period in a state of very marked dorsiflexion and the toes are also dorsiflexed. One interesting feature was observed which is not clearly brought out by the tracings. When the foot was brought forwards it made contact with the ground with the heel, and then the forepart of the foot fell to the ground with great rapidity and afterwards oscillatory movements of the toes occurred. Although these movements are not shown very clearly on the outline drawings, they were quite obvious in all the animals after all the muscles had been divided.

During the support period, the delay already mentioned in the raising of the heel from the ground is prolonged, but this actually occurred towards the end of the support period just before the foot leaves the ground. It will be noticed that the heel can still be raised from the ground even although all the muscles on the posterior aspect of the ankle have been divided. This is probably

carried on largely by the muscles of the forequarters dragging the body forward, but it shows again the point which has already been made, that when muscles are put out of action other muscles will attempt to carry on the work of those which have been lost, and the success which they achieve will depend largely on the quantity of muscle force which has been lost. The conception of muscles working as groups is very generally accepted, but it is here suggested that the whole of the musculature of the body should be looked upon as composed not of separate organs but as a continuum, united by means of their nervous connexion with the central nervous system and acting as a whole, so that, as long as the reciprocal innervation apparatus is intact, when one muscle fails another takes on its work.

RABBIT 14

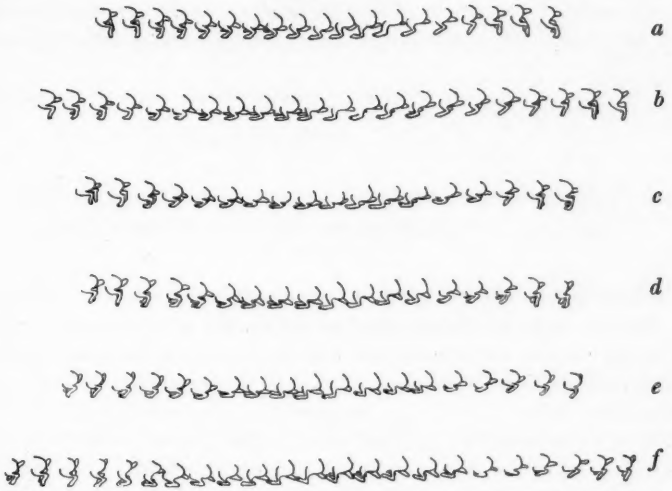

Fig. 2. Rabbit 14. *a*, Normal gait; *b*, deep flexors cut; *c*, deep flexors and plantaris cut; *d*, deep flexors, plantaris and soleus cut; *e*, deep flexors, plantaris, soleus and outer head of gastrocnemius cut; *f*, all muscles cut.

The tendons were cut in the following order:

(1) Deep flexors,
(2) Plantaris,
(3) Soleus,
(4) Outer head of gastrocnemius,
(5) Inner head of gastrocnemius.

After division of the deep flexor tendons, no alteration takes place in the movements of the ankle. The toes are held in a very slightly dorsiflexed position but this is only very slightly marked (Fig. 2*b*). After section of the deep flexors and the plantaris (Fig. 2*c*), there is little change in the movements of the ankle and none that can be perceived in the outline drawings. A careful measure-

ment, however, of the variations of the angles of the long axis of the foot and the tibia showed that the plantar flexion and the raising of the heel were slightly retarded, but this did not reveal itself on the screen. The toes, however, became markedly extended. This, of course, is to be expected, as the whole of the flexor musculature of the toes, except for the small intrinsic muscles of the foot, has been thrown out of action and therefore the extensor muscles are able to work without any opposition from antagonists. During the time the foot is on the ground, the hyper-extension is much less marked, presumably due to the extensors of the toes not being in active contraction.

Section of the deep flexors, plantaris and soleus makes no further difference to the gait. After division of the deep flexors, plantaris, soleus and the outer head of gastrocnemius, there is a definite increase in the dorsiflexion of the ankle throughout the whole of the locomotory cycle. During the support period, the length of time the foot remains on the ground is definitely increased

Fig. 3. Rabbit 12. *a, Left leg*: all muscles cut; *b, right leg*: all muscles *except soleus* cut.

and, following from this, there is a considerable delay in the raising of the heel. The toes show oscillatory movements similar to those seen in rabbit 13 after all the muscles are divided, but they are much less marked. When all the muscles had been sectioned the movements were similar to those described in rabbit 13.

Rabbit 14 was a repeat experiment of a series of operations which had been carried out in rabbit 12, but owing to some oversight in rabbit 12 the soleus on the right side was not divided. This oversight, however, gave a most interesting result because, at the end of the experiment, all the muscles of the left side had been divided and only the soleus on the right side remained intact. The movements of the two limbs are shown in Fig. 3. Note that the tracing for the right leg is to be read from left to right. These two tracings demonstrate very clearly the marked difference the preservation of the relatively small soleus makes to the movement of the limb. On the left side, where all the muscles have been divided, the movements of the limb resemble very closely the end result in the other animals, but on the right side the gait, although differing from that of the normal animal, resembles it much more closely than it does the movements of a limb in which all the muscles have been thrown out of action. This result gives further evidence in favour of the hypothesis that the muscles which remain perform extra work in an attempt to take the place of the muscles which have been lost. It has already been mentioned that the soleus is the one "red" muscle of the series, and the results of this experi-

ment would suggest that the action of "red" muscles does not differ from the action of "white" muscles.

RABBIT 11

The muscles were cut in the following order:

(1) Plantaris,
(2) Soleus,
(3) Inner head of gastrocnemius,
(4) Outer head of gastrocnemius,
(5) Deep flexors.

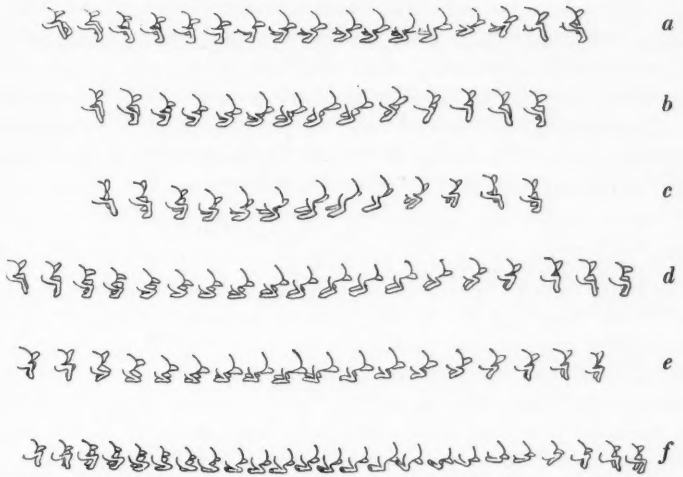

Fig. 4. Rabbit 11. *a*, Normal gait; *b*, plantaris cut; *c*, plantaris and soleus cut; *d*, plantaris, soleus and inner head of gastrocnemius cut; *e*, plantaris, soleus and both heads of gastrocnemius cut; *f*, all muscles cut.

Section of the plantaris and soleus made little difference to the movements of the ankle. After division of the flexors, soleus and inner head of gastrocnemius, the movements of the ankle during the suspense period are unaltered, but during the time when the foot is on the ground there is a delay in the raising of the heel, otherwise the movements of the ankle are normal. The toes are now being held in a slightly more flexed position, showing that the deep flexor is in a hypertonic state. After the division of the outer head of gastrocnemius, when only the deep flexor remains, there is a very striking change in the gait. The toes are now hyperflexed indicating the additional work done by the flexor muscle. This, however, is inadequate for the purpose of moving the ankle, so that when the foot reaches the ground the heel is the first portion to make contact. The raising of the heel from the ground is very greatly delayed and only occurs near the very end of the support period. After section of all the muscles the usual condition is seen, but the oscillations of the toes are better marked than in the other experiments.

In rabbits 16 and 18 the same general tendencies were observed. In rabbit 16 the muscles were cut as follows:

(1) Soleus,
(2) Deep flexors,
(3) Inner head of gastrocnemius,
(4) Plantaris,
(5) Outer head of gastrocnemius.

It was only after the division of the plantaris that there was any delay in the time of the raising of the heel from the ground. In the toes there was a very slight amount of extension which appeared to be confined to the distal phalanx, and there was no further increase in the amount of extension until after section of the plantaris.

In rabbit 18, only the inner head of gastrocnemius, deep flexors, and plantaris were divided, as the animal unfortunately died before the experiment was completed. The results in this animal, however, resemble very closely those which had been obtained in the other experiments.

SUMMARY AND CONCLUSIONS

The muscles studied in this investigation were those which are found in the calf of the rabbit. After the section of any one muscle, little or no alteration occurred in the gait of the animal. Indeed, generally, it was not until three muscles were divided that noticeable changes could be detected. Evidence is brought forward which suggests that after muscles are divided the other muscles contract more strongly and take on the work of the muscles which have been divided. It is only after a relatively large quantity of the muscles to a joint have been thrown out of action that the remaining muscles are unable to act as efficient substitutes for those which are lost and that any marked alteration takes place in the gait. It is, of course, to be remembered that the movements which have been studied are relatively simple ones carried out by large muscles, and it does not necessarily follow that the evidence can be applied without modification to muscles and joints carrying out delicate and complex movements such as are found in the human hand.

In these experiments the innervation of the muscle remained intact.

ACKNOWLEDGEMENTS

This investigation was conducted in the Departments of Anatomy and Physiology of Manchester University, and I have to thank Profs. Stopford and Raper for the facilities granted to carry out this in their departments. I am greatly indebted to Mr Gooding for valuable assistance at all stages of the research, and to Miss Dobson for preparing the manuscript for the press. The expenses incurred were met by a grant from the Manchester University Medical Research Fund.

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THE DEVELOPMENT OF THE PENILE URETHRA AND THE HOMOLOGY OF COWPER'S GLAND OF MALE SPERMOPHILE (CITELLUS TRIDECEMLINEATUS) WITH A NOTE ON THE PROSTATIC UTRICLE

By M. A. H. SIDDIQI

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The spermophile, like most other members of the family Sciuridae,[1] possesses a pair of bulbo-urethral glands, an intermediate bulbar gland, and an unpaired and central long penile duct that drains the three bulbar glands into the penile urethra. The bulbo-urethral glands, which are covered only by fascia, are spherical and lie on each side of the anus at the root of the tail. A duct, whose epithelium is folded in a complex manner, passes from the ventral surface of each gland to the dorsal surface of the caudal part of the bulb of the urethra. The two ducts unite in the midline with the duct of the intermediate gland to form a long and tapering penile duct which extends through the corpus spongiosum ventral to the urethra, into which it opens in the region of the ventral flexure of the penis.

The homologies of Cowper's glands, the median bulbar gland and the penile duct are the objects of this embryological investigation.

MATERIAL AND METHODS

Embryos of 11, 13, 17, 19, 27 and 35 mm. crown-rump length were embedded in celloidin and serial sections of 20 μ thickness were mounted and stained with haematoxylin and eosin. The sections were studied by means of graphic projections and reconstructions. The region of the developing urethra, and the associated glands and ducts noted above, were studied separately in each case. The ectodermal and endodermal areas were carefully defined and charted.

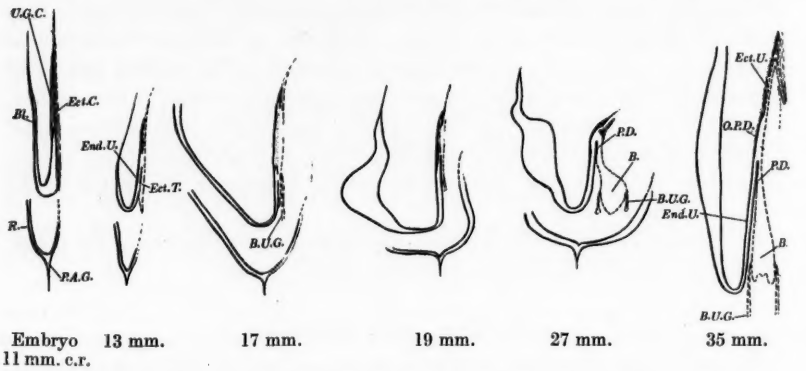
Text-fig. 2 is a diagrammatic representation of the results obtained and it illustrates my interpretation of the human homologies of the structures concerned.

DESCRIPTION

In the embryo of 11 mm. crown-rump length, the urorectal septum has completely separated the rectum from the urogenital sinus. The epithelium of the urogenital sinus extends cranially towards the allantois and ventrally to the genital tubercle where it is in the form of a solid, sagitally disposed double-layered plate of cells enclosing a potential cavity which is continuous with the

[1] The male reproductive tract of the Sciuridae has recently been studied in detail by Mossman, *et al.* (1932). Their paper includes a comprehensive review of the literature on the subject.

actual cavity of the urogenital sinus. The lower urinary tract at this stage is in the form of a U loop with the bend of the U directed caudally. The cranial end of the dorsal limb of the U terminates at the body stalk, and the cranial end of the ventral limb ends at the genital tubercle. The body stalk and the genital tubercle are very close together owing to the non-existence of a sub-umbilical ventral abdominal wall at this stage of development. The dorsal limb of the U is the allantois, and it is destined to give rise to the bladder, while the ventral limb takes part in the formation of the penile urethra. The cavity of the urogenital sinus, which is continuous around the U-shaped bend of the loop with that of the allantois, traverses only the caudal one-third of the ventral limb. Beyond this point the ventral limb is composed of the continuation of the urogenital sinus epithelium, and in apposition with the ventral surface of this a second solid mass of cells formed as it were by an infolding of



Text-fig. 1. Graphic reconstruction from a series of spermophile embryos of the posterior end of the gut and the lower urinary tract. *U.G.C.* urogenital mass of cells; *Ect.C.* ectodermal mass of cells; *Bl.* bladder; *U.G.S.* urogenital sinus; *R.* rectum; *P.A.G.* post anal gut; *End.U.* endodermal urethra; *Ect.T.* ectodermal tube; *B.U.G.* bulbo-urethral gland; *P.D.* penile duct; *B.* bulbar gland; *O.P.D.* orifice of penile duct.

the ectoderm, derived from the urogenital sinus. The potential linear cavity it encloses is continuous with the external cloaca. The endodermal and ectodermal elements of this composite structure are very easily identified under the microscope.

In embryos of 13 and 17 mm. crown-rump length the cavity of the urogenital sinus has extended farther into the ventral limb of the U, and at this stage the ectodermal plate is also canalized to form the cavity of the ectodermal cloaca. The cavities of the urogenital sinus and of the ectodermal cloaca are disposed as two parallel tubes. The caudal part of the septum separating the two breaks down, the process extending cranially towards the genital tubercle, so that the two tubes communicate freely with each other. In the 17 mm.

embryo the ectodermal tube opens to the exterior and the cavity of the urogenital sinus thus obtains an indirect external opening.

In the 19 mm. embryo the caudal communication between the two tubes is obliterated by the ingrowth of lateral folds comprising both ectodermal and endodermal layers, and the caudal end of the ectodermal tube once again becomes blind. In the 27 mm. embryo the ingrowth of the lateral folds has progressed much farther cranially. At this stage the blind caudal end of the ectodermal tube is greatly dilated to form an intermediate bulbar dilatation and has separated from the surface ectoderm. Moreover a pair of lateral ducts open into it, the ducts being those of Cowper's glands, which start developing at about the 17 mm. stage as solid outgrowths of cells from the intermediate ectodermal bulbar enlargement.

The cranial part of the ectodermal tube, which lies immediately ventral to the endodermal tube (endodermal urethra), becomes the median unpaired penile duct. The ingrowth of the lateral folds which forms the unpaired penile duct stops short caudal to the genital tubercle, so that the penile duct is provided with an opening into the urethra, as occurs in the 27 mm. embryo. The 35 mm. embryo shows that distal to the orifice of the penile duct the urethra is developed from the ectodermal tube, for the cells of the urogenital sinus do not extend beyond this level.

The development of the region can be summarized as follows: The floor of the external cloaca becomes folded cranially to form an ectodermal tube and at the same time the cavity of the urogenital sinus extends towards the genital tubercle to form an entodermal tube which lies parallel and dorsal to the ectodermal tube. The septum which separates these two tubes breaks down and the cavities of the two tubes communicate with each other. At the same time the urogenital tube acquires an indirect opening to the exterior through the ectodermal tube.

The septum which originally separated the two tubes reforms and the two tubes once again become separated except cranially where their communication persists. Thus the ectodermal tube, whose caudal end is blind, is again cut off from the urogenital sinus. Its caudal end dilates and forms the intermediate bulbar gland, while the rest of it becomes the median unpaired penile duct. The endodermal tube derived from the urogenital sinus gives rise to the penile urethra up to the orifice of the unpaired penile duct.

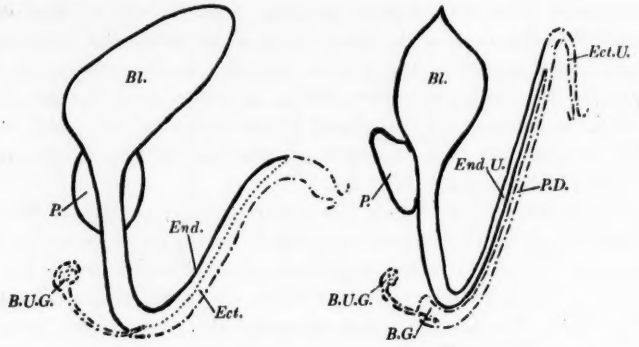
DISCUSSION

My observations lead me to agree with the views of Mossman, Lawlaw and Bradley on the homologies of the accessory glands. They write:

"So while we have for the present thought it advisable to accept the typical sciurid Cowper's glands as homologous to those of Primates and other mammals, it may be worth while to point out some of the possibilities as to the manner of development of the bulbar gland and penile duct which is what will eventually

prove or disprove our assumption. We are inclined to the belief that they are true bulbo-urethral glands and that the bulbar gland and the penile duct simply are modifications of the penile portion of their ducts. Two other possibilities immediately present themselves, however. One is that the penile duct and bulbar gland really are developmentally a urethral sinus or diverticulum, as Tulberg supposed, split off from the urethra in some manner and carrying the openings of Cowper's ducts. A second possibility is that the whole penile duct and bulbar gland with Cowper's duct and glands are actually ectodermal structures, the opening of which has become incorporated into the distal part of the penile urethra."

In spite of the danger of drawing homologies between mammals as widely separated as man and spermophiles, certain speculations on the subject appear worthy of record.



Text-fig. 2. Diagram of the lower urinary tract in man (A) and spermophile (B) to show the contribution of endoderm and ectoderm towards the formation of the penile urethra and its associated glands. The continuous line represents the endoderm and the broken line the ectoderm. *Bl.* bladder; *P.* prostate; *B.U.G.* bulbo-urethral gland; *End.* endoderm; *Ect.* ectoderm; *Ect.U.* ectodermal urethra; *End.U.* endodermal urethra; *P.D.* penile duct; *B.G.* intermediate bulbar gland.

By referring to Keibel's reconstruction as it appears in text-books, to Frazer's as it is given in his manual, and to Koff's in *Contributions of Embryology*, vol. XXIV, I find a very close analogy between man and the spermophile as to the limits of extension of the urogenital cells towards the genital tubercle. The process of development up to the formation of an ectodermal tube by the fusion of the genital folds and the manner in which the urogenital sinus forms a communication with this tube by the disappearance of the intervening septum are the same in the spermophile and in man. The process involved in the later separation of the ectodermal tube by lateral folds as occurs in the spermophile does not seem to occur in man. During the development of the human urethra, the structures that correspond with those which in the spermophile are derived from the ventrally situated ectodermal tube

(bulbar gland and the penile duct) freely communicate along their entire length with the cavity of the dorsally situated endodermal tube, which in the spermophile alone forms the penile urethra. In terms of this homological interpretation, the proximal two-thirds of the mucosa of the dorsal part of the human penile urethra is endodermal in origin, while the bulb of the urethra, Cowper's glands, the floor of the proximal two-thirds, and the whole of the terminal third of the penile urethra are ectodermal in origin. In this connexion, it would seem significant that lacunae occur on the dorsal wall but not on the floor of the penile urethra in man. The lacuna magna probably marks the anterior limit of endodermal extension.

Prostatic utricle

Careful examination of serial sections of the prostatic urethra of an adult male spermophile does not show the presence of any remains of the Müllerian ducts. Mossman, Lawlaw and Bradley who examined three such serial sections give their findings thus, "a minute prostatic utriculus similar to that of the chipmunk in position and form but with no opening was found in one animal. None at all was found in the other two series of sections".

In each embryo the Wolffian ducts closely accompany the Müllerian ducts and open into the lumen of the urethra. The Müllerian ducts in the 17 and 27 mm. embryos are in two parts. The cranial parts diverge laterally from each other, while the caudal parts lie in close apposition to each other in the midline, sandwiched, as it were, between the Wolffian ducts on either side. Very fine lumina are present for a short distance in the cranial parts of the ducts, but the caudal parts are not canalized. The cranial parts of the Müllerian duct are completely absent in the 35 mm. embryo. The caudal parts are represented by two columns of cells lying side by side.

In each of the embryos investigated, the caudal extremities of the ducts terminate abruptly and are embedded in a mesodermal mass which demarcates them from the mucous membrane of the urogenital sinus in that situation. In each of the three stages examined the distance between the terminal ends of the Müllerian ducts and the level of entry of Wolffian ducts into the urogenital sinus is 40 μ.

A comparison of the sizes of the ducts and of their histological appearance in the present series shows that the ducts begin to retrogress before the 27 mm. stage is reached and that the divergent cranial parts have completely disappeared in the 35 mm. stage and that even the caudal parts are in a markedly retrogressive state. In none of the three embryos examined did the cells of the Müllerian ducts come into contact with the cells of the urogenital sinus, nor was there any appearance of cellular proliferation analogous with the sinovaginal bulbs which are formed by the epithelium of the urogenital sinus in man (Koff, 1933).

Only one of the four adult specimens examined by Mossman and his coworkers possessed a minute utricle, and even in this case the utricle did not

open into the urethra. Its failure to do so was conceivably due to the non-formation of sino-vaginal bulbs, and it may be concluded that the normal absence of the utricle in the spermophile is due both to the non-formation of vaginal (Frazer) or sino-vaginal (Koff) bulbs, and the complete retrogression of the Müllerian ducts.

SUMMARY

Serial sections of spermophile embryos of 11, 13, 17, 19, 27 and 35 mm. crown-rump length were investigated in order to study the development of the penile urethra, the bulbo-urethral and bulbar glands and the penile duct. The conclusions arrived at are as follows:

(1) The bulbar gland, the bulbo-urethral gland and the penile duct are purely ectodermal in origin.

(2) The proximal penile part of the urethra up to the orifice of the penile duct is purely endodermal, and the distal terminal part is ectodermal in origin.

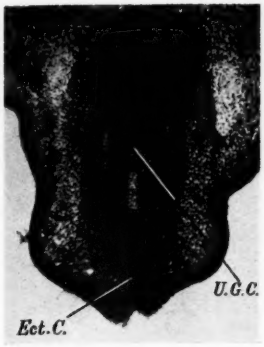
(3) A consideration of the homologies between the above-mentioned structures of the spermophile and the corresponding organs in man suggests that the human bulbo-urethral gland, the bulbar enlargement of the human urethra, and the floor of the proximal two-thirds of the urethra and the whole of the terminal one-third are ectodermal, while the roof of the proximal two-thirds is purely endodermal in origin.

(4) Müllerian ducts begin to develop between 13 and 17 mm. stage and start retrogressing in the male before the 27 mm. stage is reached.

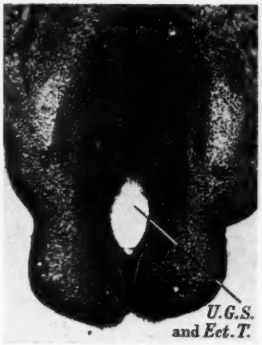
(5) Absence of a utricle in the spermophile is probably due to the non-formation of vaginal (Frazer) or sino-vaginal (Koff) bulbs associated with the complete retrogression of the Müllerian ducts.

ACKNOWLEDGEMENTS

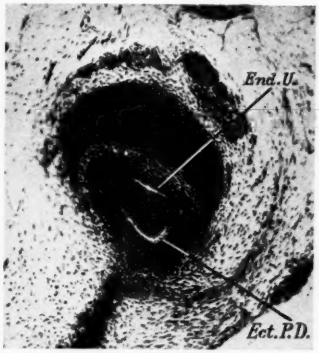
I wish to express my best thanks to Dr H. W. Mossman for sending to me the embryos and for his very useful instructions. I am also grateful to Professors J. C. B. Grant and W. H. Piersol for their hopeful interest and to Mr H. E. Lemesurier for his assistance in preparing the sections and photographs.



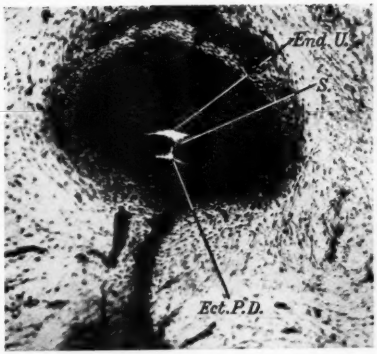
17 mm., level 1



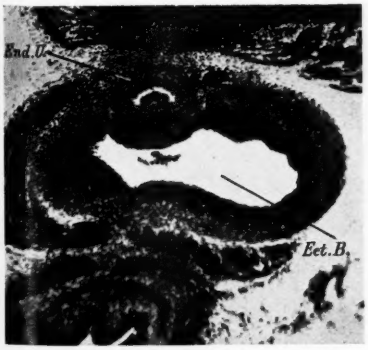
17 mm., level 2



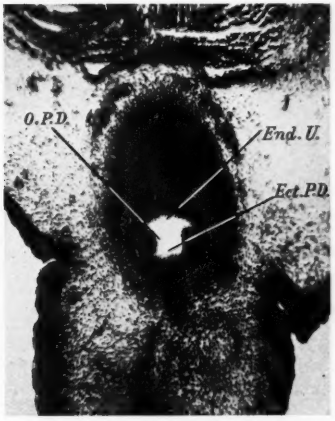
27 mm., level 3



27 mm., level 2



27 mm., level 4



35 mm., level 5

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EXPLANATION OF PLATE I

Microphotographs from serial cross-sections of the spermophile embryos. *U.G.C.* urogenital mass of cells; *Ect.C.* ectodermal mass of cells; *U.G.S.* and *Ect.T.* common cavities of urogenital sinus and the ectodermal tube; *End.U.* endodermal urethra; *Ect.P.D.* ectodermal penile duct; *S.* septum forming to separate the ectodermal tube from the urogenital sinus; *Ect.B.* ectodermal bulb; *O.P.D.* orifice of penile duct into the endodermal urethra.

A SIMPLE APPARATUS FOR STIMULATING HUMAN NERVES

By VERNE T. INMAN and ROBERT C. COMBS

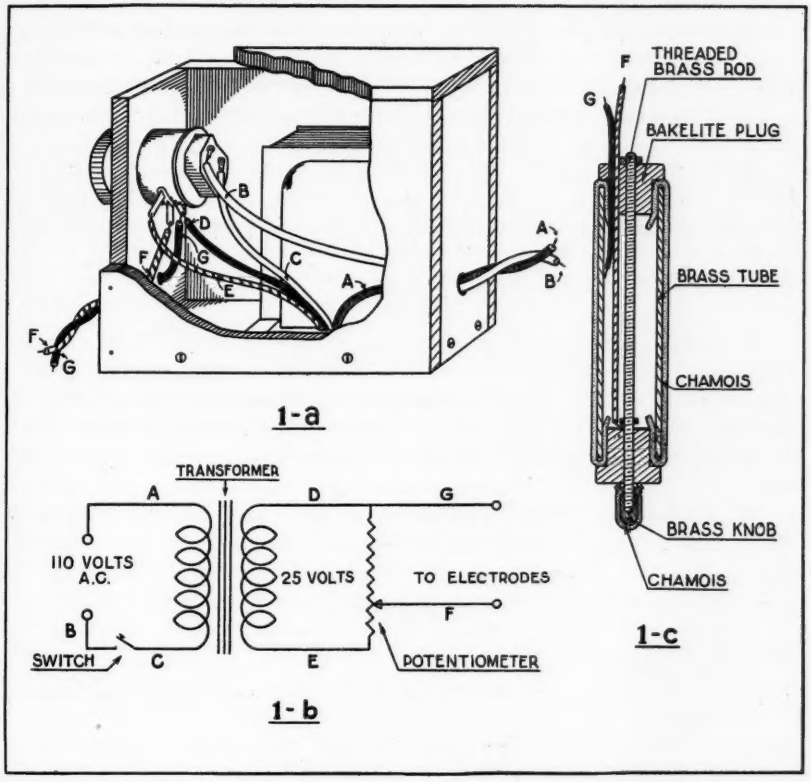
Division of Anatomy, University of California Medical School, Berkeley

Trotter & Davies (1909) ascertained the exact situation of sensory nerve trunks by stimulating them with a weak faradic current; this evoked a tingling sensation, projected into a cutaneous area supplied by the nerve. Hughson (1922) employed this method to demonstrate to students of anatomy the distribution of the cutaneous nerves, using a small induction coil (Harvard Instrument Company) energized by an ordinary dry cell; the indifferent electrode was applied to an arm or a leg, and the stimulating electrode to the skin immediately over the nerve. By stimulating the nerve trunk at an appropriate place, he showed that the area of sensory distribution could be marked out by the student from the projected tingling sensation.

The outfit described herein has been designed as an improvement on the inductorium for class use. The apparatus comprises a transformer and a potentiometer. The fixed transformer, which is of a toy or bell type, reduces the 110 V. of the A.C. supply to 25 V. The potentiometer is a common radio filament control rheostat of 50,000 ohms resistance, combined with an on-and-off switch controlled by the same knob. In Figs. 1*a* and 1*b* are shown a sketch and a wiring diagram of the unit; for the sake of clarity these are labelled alike. One lead (*A*) of the 110 V. passes directly to the primary of the transformer; the other (*B*) passes to one pole of the on-and-off switch, then continues as *C* from the other pole of the on-and-off switch to complete the circuit of the primary coil. From the secondary of the transformer leads *D* and *E* are connected through the potentiometer. Wire *F* goes from the variable pole of the potentiometer to one electrode, while *G* goes from the fixed pole of the potentiometer to the other electrode. By means of the potentiometer the current delivered to the electrodes can be varied from zero to a maximum of 25 V.; it is so arranged that when the potentiometer has reduced the voltage to zero further turning of the knob opens the on-and-off switch in the primary circuit. The entire apparatus is compactly mounted in a small wooden box measuring $4 \times 4\frac{1}{2} \times 5$ in.

To eliminate the inconvenience of using a separate indifferent electrode which must be strapped to an arm or a leg, we have designed a single instrument which combines both the indifferent and the stimulating electrodes (Fig. 1*c*). It consists of a brass tube 4 in. long and 1 in. in diameter covered

with chamois which is held in place at each end of the tube by two bakelite plugs. The bakelite plugs are securely held by a threaded brass rod passing through their centres and fixed by a nut at each end. One end of the rod is fitted with a ½ in. brass knob covered with chamois. The two leads from the secondary of the transformer pass into the tube through a hole in one bakelite



Figs. 1*a* and 1*b*. Sketch and wiring diagram, respectively, of apparatus for stimulating nerves; for explanation of lettering, see text. Fig. 1*c*. Diagram of combined stimulating and indifferent electrodes; for description, see text.

plug, and are soldered, one to the inside of the brass tube, the other to the brass rod. When the instrument is used, the chamois-covered brass tube serves as the indifferent electrode, being grasped by the palmar surface of the hand and digits; the chamois-covered brass knob is the stimulating electrode.

Before using the instrument the chamois surfaces are moistened with saline. The stimulating electrode is applied over the nerve trunk and the current is increased by turning the knob until a tingling sensation is felt in the cutaneous area supplied by that nerve. Little or no sensation is experienced

in the hand grasping the indifferent electrode, because of the large area of contact.

When a mixed nerve, such as the median or the ulnar, is being studied, strong stimulation elicits instructive muscular contractions; such strong currents, however, are apt to produce tingling in the hand grasping the electrode.

To obviate confusion, it may be pointed out that this method of studying nerve areas by the projection thereinto of a tingling sensation is not identical with that used by Thompson *et al.* (1933, 1934) in their study of nerve areas: in the latter work, although the nerves were stimulated with an alternating current, the areas were outlined not on the basis of the tingling sensation, but on that of their insensibility to touch. This insensibility is sometimes noticed (if tested for) when using the apparatus described above; but the tingling is easier for students to observe, and serves the purpose of routine class instruction.

SUMMARY

A simple electric outfit is described wherewith students may easily experience a vivid subjective demonstration of the principal cutaneous nerve areas of the body. It is inexpensive, noiseless, compact, portable, sturdy and may be "plugged in" to any 110 V. A.C. outlet. Doubtless modifications could be devised for use with other varieties of current from the main.

We gladly acknowledge the assistance of Mr J. H. Wiens; his suggestion that we use a potentiometer was especially valuable.

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ON SUBCUTANEOUS VEINS OF THE NECK

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Head of the Faculty: Professor J. B. Zeldowitch

In this work, based on the study of 96 subjects, I hope to give accurate numerical data concerning the frequency of variation in their courses and direction, their branches and their anastomoses, of the subcutaneous veins of the neck.

VENA JUGULARIS ANTERIOR

The initial branches of the vena jugularis anterior are tiny subcutaneous veins in the middle part of the neck, rarely anastomosing with the internal jugular veins. The anterior jugular vein joins the vena subclavia on its own side medial to the entrance of the vena jugularis externa. This, the usual description, has been accepted by several authors (Henle, Luschka, Rauber, Quain, Poirier & Nicolas, Spalteholz and others).

The special work of Sebileau & Demoulin on the veins of the neck contains numerical data concerning the different points where this vein takes its origin. According to my observations this vein emerges from the superficial jugular veins only in 44 cases (22·9 per cent), its initial branches beginning either in:

(1) The submental region as thin subcutaneous veins lying parallel to one another at some trifling distance from the central line of the neck (Fig. 1) and so descending to the fossa jugularis where they sometimes form an anastomosis-arcus venosus juguli: at other times they anastomose a little higher up or on a level with the prominentia laryngea, or they do not anastomose at all. Twenty-four such non-anastomosing veins occurred (12·5 per cent).

(2) Besides the origin of the vena jugularis anterior just described, I have observed that it takes its origin from the subcutaneous veins in the anterior region of the neck, i.e. in the trigonum colli mediale (Fig. 2). In addition to the trifling anastomosis in the submental region, there were subcutaneous branches along the anterior margin of the m. sterno-cleido-mastoideus and others collecting blood from the small subcutaneous veins of the internal triangle of the neck, which united together and followed the usual direction to the vena subclavia on its own side. This arrangement was observed 20 times, or 10·4 per cent.

In addition to the above the following were observed:

In 27 cases	the vena jugularis anterior received branches arising from	vena jugularis externa.
In 6 cases		vena jugularis interna.
In 1 case		vena thyreoidea superior.
In 34 cases		vena facialis anterior.
In 17 cases		vena facialis communis.
In 3 cases		vena facialis posterior.
In 20 cases		glandula parotis.
In 19 cases		glandula submaxillaris.
In 8 cases		isthmus glandula thyreoidea.

Short venous trunks coming from adjacent regions open into this vein; thus

(1) from deep layers of trigonum caroticum in 49 cases,

(2) from regio submentalis in 22 cases,

(3) from the region of trigonum omotrachealis in 9 cases.

I particularly noted all the venous trunks, which entered the anterior jugular veins; in several cases instead of one trunk, several venous trunks entered the anterior jugular veins; they emerged from different regions of the neck, and this is why their total sum resulted in figures exceeding 100 per cent. The anterior jugular trunks themselves were arranged as follows—only in 39 cases (40 per cent) did they follow the textbook pattern, i.e.

(1) Parallel straight venous trunks lying beside the medial plane: (*a*) of equal size, on both sides and symmetrically placed (Fig. 1); an inferior or a superior anastomosis may be present, or they may be completely independent of each other. Parallel uniform anterior jugular veins were observed 16 times (16·6 per cent). (*b*) In 23 subjects (23·9 per cent) the anterior jugular veins were identical in course but differed in size. (*c*) In 18 per cent the right was the larger. (*d*) In 5 (2 per cent) the left was the larger (Fig. 4).

(2) In 10 (10·4 per cent) the disposition of the anterior jugular veins was different; their course lay near the medial border of the m. sterno-cleido-mastoideus which they followed: also their origins were dissimilar. Out of these 20 anterior jugular veins (Fig. 5):

6 venae jugulares anteriores emerged from the glandula parotis together with vena jugularis externa.

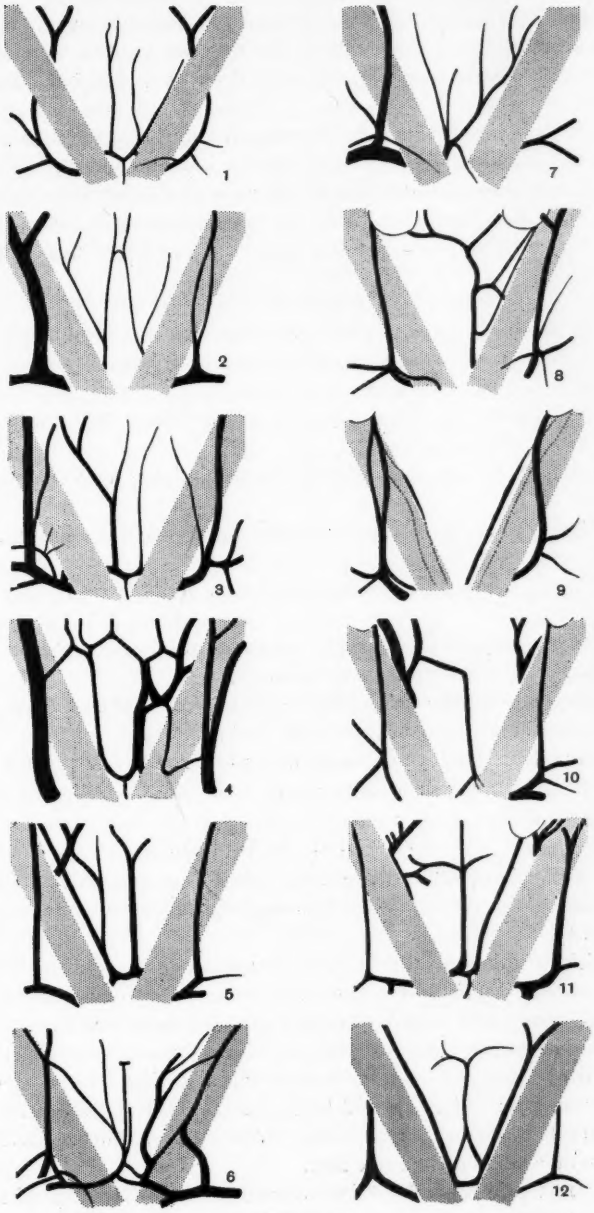
4 venae jugulares anteriores emerged from the glandula submaxillaris.

5 venae jugulares anteriores were the direct continuation of the vena facialis communis.

4 venae jugulares anteriores began from the vena facialis anterior.

1 vena jugularis anterior departed from the vena jugularis interna on the level with the upper third part of the m. sterno-cleido-mastoideus.

(3) In 18 subjects (18·7 per cent) I observed a multiple origin of the anterior jugular vein (Fig. 6) from the subcutaneous and from the deep veins of the neck as well; many small venous trunks were to be observed on both



Figs. 1–12

sides in the vicinity of the trigonum colli mediale in the medial or lower part of the neck. These veins unite so as to form two anterior jugular trunks.

(4) In 13 subjects (13·5 per cent) the anterior jugular vein was single; in 11 cases the right one only was present (Fig. 7), and in two cases the left one only.

(5) Three subjects were absolutely without anterior jugular veins on either side (Fig. 9); no small subcutaneous venous branches in the region of the middle triangle were present. The lower ends of the internal jugular veins were very near each other, but there was no anastomosis between them.

The external jugular veins are of average size and follow their usual course.

Tributaries entering the vena jugularis anterior

The anterior jugular vein receives branches from the neighbouring regions both from deep as well as superficial tissues. The tributaries of 140 (72·9 per cent) anterior jugular veins were enumerated.

(1) 51 (26·5 per cent) anterior jugular veins coming from the deep layers of the neck had side branches:

(*a*) 29 times (15·1 per cent) they were veins deriving from the vena facialis anterior.

(*b*) 4 times (2 per cent) they were veins deriving from the vena facialis communis.

(*c*) 18 times (9·3 per cent) they were veins deriving from deep layers of the neck.

(2) 50 anterior jugular veins (26 per cent) received only thin branches coming from the superficial parts of the neck.

(3) 39 anterior jugular veins (20·3 per cent) had branches emerging from superficial as well as deeper layers of the neck.

(4) A complete absence of side branches in the anterior jugular vein was to be observed 46 times, or in 24 per cent of the cases.

VENA MEDIANA COLLI

The vena mediana colli or the central vein of the neck which lies along the linea mediana colli and which is rarely found, was observed by me in 6 subjects (6·2 per cent).

The origin of this central vein in 5 cases was in the regio submentalis; in one case this vein (Fig. 10) branched off from the vena facialis anterior dextra.

In three cases these veins entered the vena subclavia sinistra after passing behind the m. sterno-cleido-mastoideus; once this vein entered the vena subclavia dextra joining with the end of the vena jugularis externa dextra near its termination (Fig. 11); and twice the trunk of the odd vena mediana colli was divided into two large venous trunks each of which opened into the anterior jugular vein on the same side.

This central vein took the following course: in two cases (Fig. 12) it ran for a short distance along the central line of the neck, and divided into two

trunks of almost the same size as the original vein; these then joined with the anterior jugular veins. In one case (Fig. 13) the vena mediana colli lay along the middle line of the neck and had no side branches except a considerable anastomosis with the vena facialis anterior dextra, which opened into the internal jugular vein. In one case the vena mediana colli (Fig. 11) anastomosed with the vena thyreoidea superior dextra and was accompanied by an anterior jugular vein, present only on the left side. The vena mediana colli (Fig. 14) which carries the blood from the regio submentalis receives also a large vein from the glandula parotis sinistra.

VENA JUGULARIS EXTERNA

The vena jugularis externa or vena jugularis externa posterior or vena cutanea colli posterior emerges always from the substance of the glandula parotis, and descends under cover of the m. platysma over the external surface of the m. sterno-cleido-mastoideus lying in the lower lateral third of the neck and passing through the deep layers to enter the vena subclavia.

The different origins of the vena jugularis externa were:

(1) 4 times it commenced from the venae faciales anterior et posterior (Fig. 15).

(2) The vein appeared from under the lateral border of the m. sterno-cleido-mastoideus, 5 times on the right and 4 times on the left; in 3 cases out of 9 this occurred on both sides, in 3 other on one side only: twice on the right and once on the left side (Fig. 1).

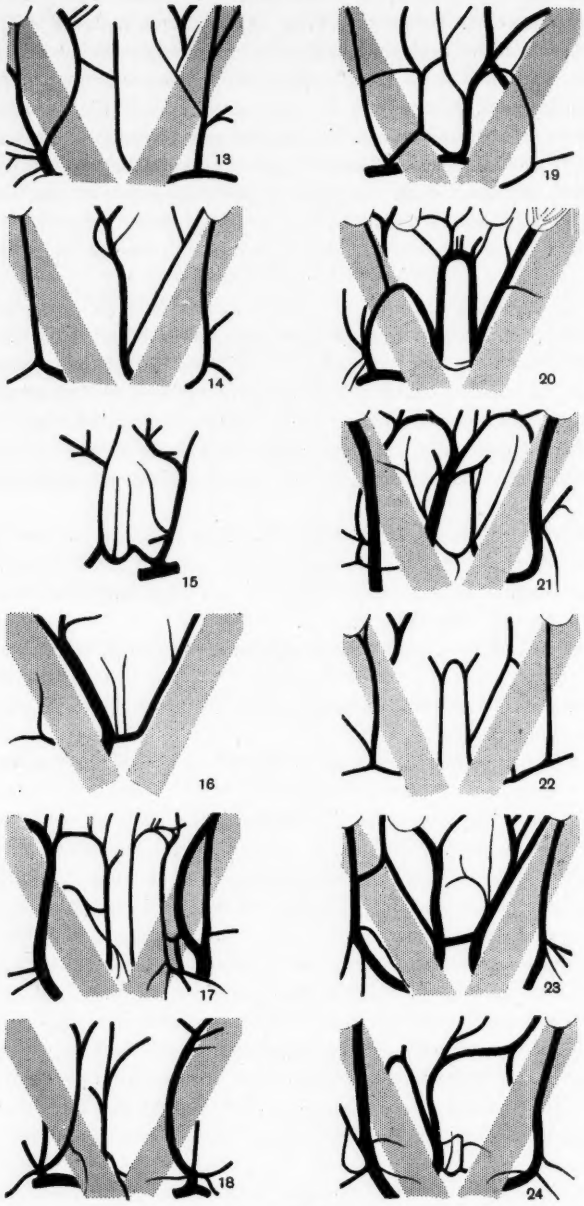
(3) In 2 cases the vena jugularis externa was completely absent on the left side.

(4) In one case the vena jugularis externa started from the substance of the submaxillary gland.

The following peculiarities were observed in the disposition of the vena jugularis externa:

(1) In 2 cases the vein (Fig. 16) emerging from the glandula parotis ran along the lateral border of the m. sterno-cleido-mastoideus towards the trigonum colli mediale to the fossa jugularis; in the fossa jugularis between these veins was noticed a large anastomosis into which opened also two thin anterior jugular veins. The trunk of the vena jugularis externa is usually single, but double trunks occurred in 15 cases (7·8 per cent): such double trunks occurred on the right side in 6, on the left side in 9 cases. When such double trunks were found, two independent veins emerged from the glandula parotis. These veins either opened separately into the vena subclavia (6 cases) (Fig. 17), or (Fig. 3) the two trunks united to form one vein (9 cases) just below where the branches of the plexus cervicalis appear behind the m. sterno-cleido-mastoideus. This single vein opened at the usual point into the vena subclavia.

(2) In 12 cases (6·2 per cent) division of the venae jugularis externae trunk on the right side was observed 3 times, on the left side, 9 times. The single



Figs. 13–24

trunk of the external jugular vein emerging from the thick layers of the glandula parotis, and divides into two trunks of somewhat lesser size than the original one directly after leaving the gland. Both trunks descend together along the external surface of the m. sterno-cleido-mastoideus and unite again below the cutaneous branches of the cervical plexus into one trunk of the same diameter as before the division.

Numerous veins from neighbouring superficial as well as deeper tissues join the external jugular vein, which receives also anastomotic branches of the anterior jugular vein. In 29 cases (15·1 per cent) the veins which joined the jugular external vein emerged from the deeper tissues.

(1) 23 times (12 per cent) they were derived from the vena facialis anterior. On the left side of the neck a short middle-sized branch of the external jugular vein, emerging from the medial aspect of the glandular parotis below the angle of the jaw, entered the vena facialis anterior, this being of similar size to the vena jugularis externa.

(2) Twice the vena jugularis externa was seen to join the vena facialis communis. The vena facialis communis connected with the vena jugularis interna, and in the upper third part of the m. sterno-cleido-mastoideus entered the vena jugularis externa (Fig. 15).

(3) In 4 cases these deep branches were trunks, arising from the glandula submaxillaris (Fig. 8). A short trunk, without any side branches and of the same size as the vena jugularis externa, was seen to emerge below the mandibulae angle from the glandula submaxillaris and enter the vena jugularis externa, which took its origin from the glandulae parotis.

The superficial lateral branches of the external jugular veins in 154 cases (79·1 per cent) could be subdivided into the three following groups:

(1) Veins from the anterior part of the neck reached the external jugular veins in 33 cases (17·2 per cent). These veins, their number varying from 1 to 3, gathered the blood in the region of the anterior triangle of the neck and entered at different levels the external jugular vein.

(2) 64 external jugular veins (33·3 per cent) received tributaries from the lateral and posterior surface of the neck. These veins, like the veins of the preceding group, were of different size but never exceeding the size of the trunk of the vena jugularis externa itself. They opened at different levels into the vena jugularis externa.

(3) 55 external jugular veins (28·6 per cent) received branches from the anterior, posterior and lateral surfaces of the neck. In 38 cases (20 per cent) the external jugular vein had neither branches nor anastomoses (Fig. 5).

In these cases the vena jugularis externa took its origin from under the lower margin of the glandula parotis, and followed its course along the external surface of the m. sterno-cleido-mastoideus parallel with the nervi auricularis magni, crossing the lateral margin of the muscle below its middle; the vein followed its course without receiving any branches.

The external jugular vein anastomoses rather frequently with the adjoining

anterior jugular vein. Such anastomoses were observed in 54 cases (28·1 per cent). These connecting venous trunks pass under cover of the m. subcutaneous colli at different levels along the external surface of the m. sterno-cleido-mastoideus.

(1) Very rarely, only in 4 cases, the anastomosis between the vena jugularis externa and vena jugularis anterior was to be observed at the upper part of the above-mentioned muscle (Fig. 4). From the upper third of the external jugular vein a venous trunk, smaller than the vein itself, ascends obliquely and joins the most lateral initial branch of the anterior jugular.

(2) More frequently, in 17 cases (9 per cent), the anastomosis was seen in the lower part of the m. sterno-cleido-mastoideus (Fig. 19). In front of the lower third of the m. sterno-cleido-mastoideus passes either a straight or an arched venous trunk, which unites the ends of the external and the anterior veins.

(3) The most frequent, in 33 cases (17·1 per cent), anastomoses between the external and the anterior vein is by veins which cross transversely the middle part of the m. sterno-cleido-mastoideus.

ANASTOMOSES BETWEEN THE VEINS OF OPPOSITE SIDES

Between the anterior jugular veins a transverse venous anastomosis occurs at different levels. The anastomosis, which occurs in the region of the fossae jugularis (the arcus venosus juguli), is the one usually mentioned in textbooks. Different authors, including Sebileau & Demoulin, mention one other less frequent anastomosis between the upper parts of the anterior veins by means of transverse vessels, in front of the membrane thyreo-hyoidea. The frequency of this anastomosis was not mentioned by the authors. In 56 cases (58·3 per cent) an anastomosis occurred between the anterior jugular veins in the lower part of the neck above the incisura jugularis by means of a vein, usually called arcus venosus juguli.

(1) In 34 cases (17·5 per cent) only one anastomosis occurred between the anterior jugular veins.

(2) In the remaining 22 cases (11·5 per cent) the anastomosis was double, i.e. beside the anastomosis in the lower part there was one other transverse vessel.

(*a*) In 2 cases the arcus venosum juguli was accompanied by a second anastomosis also in the lower part of the neck, but lying a little higher than the first (Fig. 6).

(*b*) In 10 cases the anastomosis of the lower part, the arcus venosus juguli was accompanied by an anastomosis in the upper part, the upper anastomosis being at the level of the laryngeal prominence (Fig. 20).

(*c*) In 9 cases (Fig. 4) a second anastomosis was present in the hyoid region.

(*d*) In 1 case the anastomosis occurred a little lower than the laryngeal prominence.

In 9 cases (5·5 per cent) the anastomosis between the anterior jugular veins in the lower part of the neck was absent, and only the anastomosis of the upper part was present.

In 4 cases this upper anastomosis was above the laryngeal prominence (Fig. 2), in two at the same level, and in three just below (Figs. 22, 23).

In 24 cases (25 per cent) there were no traces of anastomoses between the veins of the right and those of the left side:

In 3 cases the anterior jugular veins were absent (Fig. 14); in 2 cases the anterior jugular vein was present only on the left side (Fig. 8); in 3 cases the vena mediana colli was present and opened into the vena subclavia sinistra (Fig. 9); in 6 cases two parallel anterior jugular veins were present without any connecting vessels between them.

In 6 cases small veins from the left side entered the anterior jugular vein of the right side, but the vena jugularis anterior on the left side was absent (Fig. 24). In one case I noticed in the lower part an anastomosis between two anterior jugular veins, which were connected with some branches of the vena mediana colli (Fig. 11).

CONCLUSIONS

1. The cutaneous veins of the neck are not only anastomosing, but also collecting trunks.

2. Notwithstanding the variability of the cutaneous veins, a certain regularity in the course of the superficial jugular veins can be affirmed.

3. A complete absence of the different veins is very rare.

4. The vena mediana colli is a very infrequent vessel (6 per cent).

5. In 40 per cent of the cases studied, the picture of the cutaneous jugular veins corresponded to the usual elementary textbook description.

I am happy to express my thanks to Prof. J. B. Zeldowitch for the theme given and the kind guidance obtained from him.

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A VALVED TRANSVERSE SEPTUM IN THE FEMORAL VEIN

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The venous anomaly described in this report was observed in the course of a post-mortem examination conducted by one of us (A. G. G.) in the Radcliffe Infirmary on a woman aged 50, who had died 14 days after a hysterectomy from pulmonary embolus. A clot 37 cm. long was found in the right auricle, extending into and blocking the right pulmonary artery. A search for the source of the embolus determined that it came from the veins of the left thigh, where some of the branches of the internal saphenous vein showed ante-mortem clot.

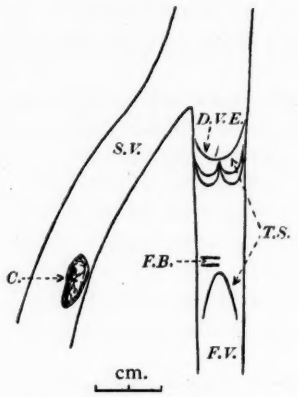

Fig. 1. *F.V.* femoral vein, and *S.V.* internal saphenous vein, both laid open. *C.* ante-mortem clot in saphenous vein. *D.V.E.* entry of deep vein into femoral vein. *T.S.* transverse septum in femoral vein, with a bicuspid valve at its proximal end, and with *F.B.* a fibrous band, adherent at either end to the septum.

In laying open the femoral vein, it was found that a transverse septum, about 6 cm. long at its attachment to the vein wall, divided the main vessel into two. At the proximal end of the superficial surface of the septum was a bicuspid valve, and at the distal end a small fibrous band (see Fig. 1). Towards the proximal end of the septum, on its other surface, was one cusp of a bicuspid valve; the second cusp was situated opposite it on the main wall of the vein.

In view of the history of recent thrombosis and of the varicose veins present in the patient's left leg, it seemed possible that the septum might be

explained on the basis of a previous thrombosis which had left it in the process of healing. This explanation was, however, contra-indicated for the following reasons. Sections taken from the femoral vein, at the level of entry of the saphenous vein and just proximal to the septum, and also slightly distal to the septum, showed no signs of old thrombosis, though such signs were present in a section taken from the saphenous vein, about 4 cm. distal to its entry into the femoral vein.

Secondly, there is no evidence to show that venous valves can be formed afresh after birth (2, 3). It may be assumed, therefore, that the septum was formed during embryonic life, for the first valves in the peripheral veins of the leg arise slightly after the middle of the fourth month (4). The most probable explanation of the septum, under the circumstances, is that it resulted from the incomplete fusion in this region of two veins during embryonic life.

Except as sequelae to thrombosis and subsequent organization, transverse septa in the leg veins have received little attention. Houzé de l'Aulnoit (1), in 1854, wrote of such structures, varying in length from 0·5 to 3 or 4 cm., in the tibial veins, but gave no suggestion as to the time or manner of their formation. "Ce sont", he said, "de véritables anastomoses longitudinales, contenues dans l'intérieur du vaisseau, au lieu de lui être extérieures."

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ANATOMICAL NOTES

A NOTE ON SCARPA'S FASCIA

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Scarpa's fascia is necessarily divided in the performance of a laparotomy below the umbilicus. It is daily seen and recognized by surgeons, yet, in the operating theatre, it is not found to conform to the orthodox anatomical description which is generally being taught.

This portion of the superficial fascia of the anterior abdominal wall below the umbilicus is fibrous and non-fatty. It is commonly said to lie deep to the superficial, fat-bearing layer of Camper, and to be loosely connected by areolar tissue to the aponeurosis of the obliquus externus abdominis.

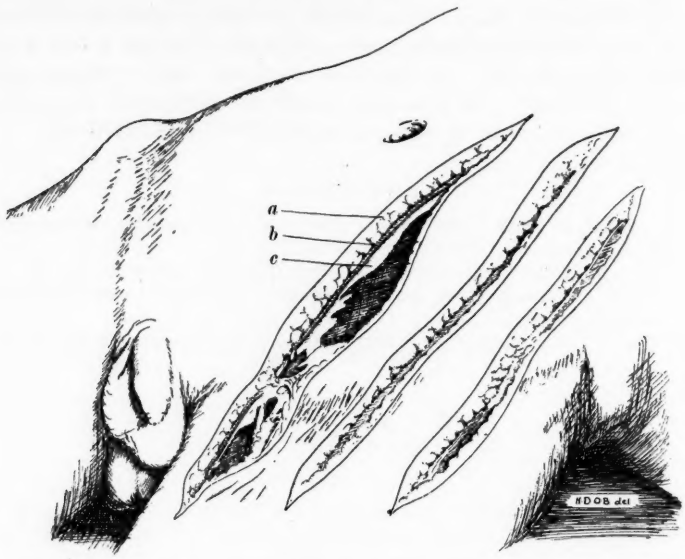

Fig. 1. Three vertical cuts in anterior abdominal wall. The medial and lateral incisions pass down to the aponeurosis of the external oblique; the middle one only passes down to Scarpa's fascia. Notice variation in gape of wound according to whether Scarpa's fascia is cut or not. *a* represents Camper's fascia, which has retracted to a greater extent than *b* (Scarpa's fascia); *c* indicates fibro-fatty layer deep to Scarpa's fascia.

Glancing at the cut edge of the structures divided by a simple incision exposing the aponeurosis in this region, one recognizes successively (1) skin; (2) a fat-bearing layer; (3) a fibrous, tough membrane (fascia of Scarpa); (4) another layer of fat, less thick than the similar and more superficial layer; (5) deep fascia which covers the external oblique muscle and its aponeurosis. Thus interpreted, Scarpa's fascia is therefore sandwiched in between two layers of fat.

The accompanying drawing is that of a normally developed subject, a boy 14 years of age, chosen to demonstrate the above. The medial of the three vertical incisions has exposed the aponeurosis, and natural retraction of the tissues, without the aid of further dissection, has produced the gaping of the wound. The more superficial fat-bearing layer has retracted to a greater extent than the deeper Scarpa's fascia, and the latter therefore is seen as a slight shelf-like projection in the side of the wound. A fatty layer deep to that of Scarpa is quite evident. The middle incision was made down to, but not through, Scarpa's fascia; it will be at once noted that the gaping of the wound is much less pronounced. In the lateral incision, the same fascia is definitely present, although slightly less well developed.

Scarpa's fascia begins almost immediately below the umbilicus. Traced downwards, it passes over Poupart's ligament, to become lost in the trabeculae enclosing the fat of the superficial fascia in the thigh. Surgeons suture its cut edges during the closure of an operation wound, knowing that, if they omit to do so, the resulting scar will eventually become needlessly wide and unsightly.

Experience in the dissection room has shown that students, misled by their textbooks, fail entirely to find this fascia of Scarpa. It may be very easily demonstrated by the above simple method.

For the accompanying illustration my sincere thanks are due to Dr H. D. O'Brien, Department of Anatomy, McGill University.

M. TENSOR LIGAMENTI CRUCIATI CRURIS (?)

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This anomalous muscle was found in the sole of an amputated left foot from a male patient and, therefore, whether or not a similar anomaly is present in the right side was not known.

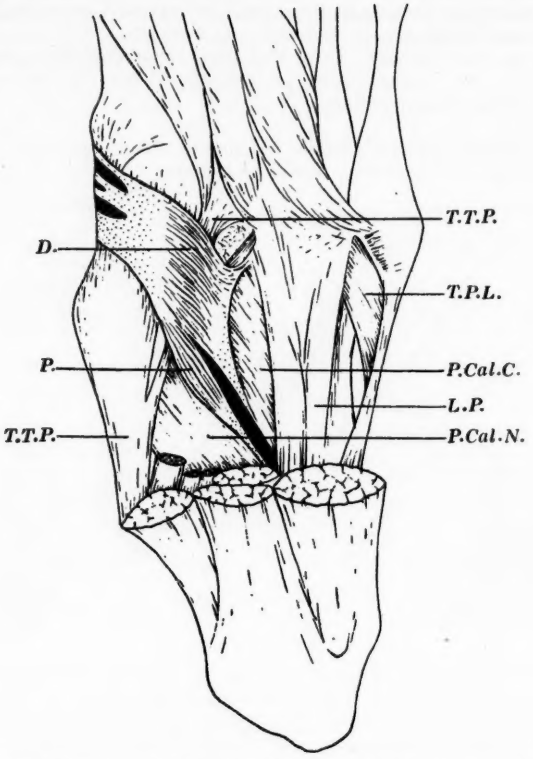

Fig. 1. Sketch of the anomalous muscle. *D.* distal part and *P.* proximal part of this muscle; *T.T.P.* tendon of tibialis posterior; *T.P.L.* tendon of peroneus longus; *P.Cal.C.* plantar calcaneo-cuboid lig.; *L.P.* plantar lig.; *P.Cal.N.* plantar calcaneo-navicular lig.

This small muscle was quadrilateral in outline and possessed two heads of origin. The proximal one was a slender, tendinous cord arising from the lateral part of the plantar surface of the plantar calcaneo-navicular ligament. As it passed medially and forwards for half an inch, its place was taken by a small spindle-shaped muscle

belly which, after crossing over the lateral part of the tendon of the tibialis posterior, joined the distal part of the muscle at the lateral side of the first cuneo-navicular articulation. The distal part was broader and was separated from the proximal one by a triangular interval. This part arose partly by tendinous and partly by muscular origin. The tendinous part arose from the under surface of the medial border of the plantar calcaneo-cuboid ligament, while the muscular origin was attached to the under-surface of the medial border of the cuboid behind the tuberosity. The fibres of both heads were directed medially and slightly forwards. As they passed over the first cuneiform bone they were represented by a fenestrated aponeurotic membrane which was continuous with the distal limb of the cruciate ligament. Judging from its possible action the above name has been suggested.

AN UNUSUAL VARIATION OF THE PHARYNGEAL MUSCLES

By T. K. CHI

Department of Anatomy, National Medical College of Shanghai, China

THE anomalies of the pharyngeal muscles are usually found in the longitudinal muscles. They may appear as muscular strand arising from the base of the occipital bone or the petrous portion of the temporal bone. The variations in the constrictors are, however, less frequently encountered. The following case is a pair of anomalous muscles found on the posterior aspect of the pharynx, forming a portion of the constrictors with their origin from the medial surface of the angle of the mandible. As their presence has not been reported in the literature, a brief account is, herewith, given.

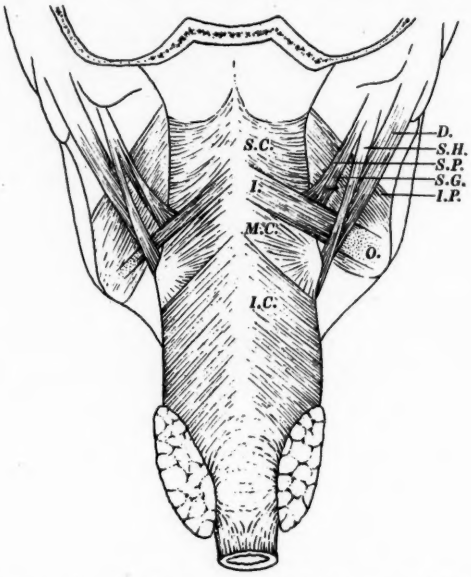

Fig. 1. Sketch of the anomalous muscles. *O.*, *I.*, origin and insertion of the right muscle; *S.C.*, *M.C.*, *I.C.*, sup., mid. and inf. constrictors; *D*, digastric m.; *S.H.*, stylo-hyoid m.; *S.P.* stylo-pharyngeus m.; *S.G.*, stylo-glossus m.; *I.P.* internal pterygoid m.

The specimen was obtained from a male body 30 years of age. The muscle of the right side was much stouter than that of the left side. It took origin from the medial surface of the angle of mandible by a number of tendinous strands some of which passed out between the fasciculi of the internal pterygoid muscle. They fused to form a fascial membrane directed upwards and medially and were covered by the posterior portion of the submaxillary gland. Underneath the digastric and stylo-hyoid muscles this fascial membrane was replaced by the fleshy fibres which formed a strap-shaped

band lying in front of the external carotid artery, immediately above its external maxillary branch, and crossing, at a right angle, the lower portions of the stylo-glossus and stylo-pharyngeus muscles. On reaching the pharyngeal wall it was blended and parallel with the upper part of the middle constrictor of the pharynx and inserted into the median raphe. Its muscular portion measured 35 mm. in length and 10 mm. in maximum width at the medial border of stylo-hyoid muscle, and its fascial portion measured 15 mm. in length, the width of which could not be well determined.

The left muscle bore the same relationships with its surroundings as the right one although it was much more attenuated.

As the origin of this pair of muscles was lateral to the pharynx, they could not be considered as one of the constrictors, but, on the contrary, rather a lateral dilator and depressor in their action.

REVIEWS

Principles of Full Denture Prosthesis. By E. Wilfred Fish. 3rd edition. (London: John Bale, Sons and Curnow, Ltd.) 1937. Pp. ix + 116, 48 figs. Price 12*s.* 6*d.* net.

This short book opens with a chapter upon the principles of denture prosthesis, and then in further detail considers three aspects that govern the stability of the artificial denture. Of these three, the occlusal contact, the adjustment of the alveoli and the palate as well as the upper jaw, and the musculature of the tongue and cheek, it is the last which is of most interest to anatomists because it involves a consideration of the muscles of the face. Just outside the corner of the mouth there is a common meeting point for all the muscles which govern the shape of the oral cavity.

The author has stated in an interesting and lucid fashion the bearing of anatomy upon the special problem of denture prosthesis.

Handbuch der Vergleichenden Anatomie der Wirbeltiere. Herausgegeben von Louis Bolk, Ernst Göppert, Erich Kallius, Wilhelm Lubosch. (Berlin: Urban und Schwarzenberg.) 1936. Band III. Darmsystem—Atmungssystem. Mit 981 zum Teil mehrfarbigen Abbildungen im Text, xi + 1018 Seiten. Bei Einzelbezug, RM. 138; gebunden, RM. 132.

Band IV. Skelettsystem I. Mit 696 zum Teil mehrfarbigen Abbildungen im Text, xii + 1016 Seiten. Bei Einzelbezug, RM. 126; gebunden, RM. 132. Für Bezieher des ganzen Werkes, RM. 115; gebunden, RM. 160.

The third volume of this handbook of vertebrate anatomy is devoted to an exhaustive survey of the various parts of the alimentary canal and the organs developed from the primitive gut. The chapters are arranged so that each division of the digestive tube is considered separately, followed by chapters on the liver, pancreas, respiratory system and the coelom. Each chapter is essentially a review of modern knowledge of the particular region or organ, the material being arranged systematically under the several vertebrate groups. Almost 200 pages are devoted to a consideration of the teeth, mouth cavity and its glands. The chapter on the teeth set out the major facts of dental morphology. An excellent chapter on the embryology of the gut in cyclostomes, fishes and amphibia by Rauther reviews the great mass of literature on the pharyngeal region of these animals. This is followed by an interesting survey of the development of the pharynx and the various epithelial organs associated with its walls. The anatomy of the adult condition is considered in detail and illustrated with many original drawings. The high standard set in the early chapters is well maintained.

The axial and visceral skeletal apparatus and the cranium are treated in great detail in the fourth volume. Prof. Remane of Halle has written the section on the vertebral column. The matter is treated in several subchapters each dealing with a single vertebrate group and preceded by a chapter on general morphology. The

sections devoted to the cranium and visceral skeleton are arranged in a manner which facilitates easy reference. Each vertebrate group is treated separately. General discussions which are kept concise are separated as short prefaces to the section devoted to a particular group of animals. A brief chapter on general skull and visceral skeleton morphology by Marinelli incorporates much of Goodrich's work. It is attractively written and provides a good survey of the subject. The chapter on the mammalian cranium and visceral skeleton by Stadtmüller is one of the most satisfying in this volume. It is exhaustive, well documented and easily consulted. Full account is taken of modern embryological work, particularly pleasing is the use that has been made of Fawcett's contributions to skull morphology in mammals. Illustrations are mostly simple line drawings well labelled and clear.

BOOKS RECEIVED

Pocket Atlas of Anatomy. By V. Pauchet and S. Dupret. 3rd edition. (Oxford University Press) 1937. Pp. xiii + 368, 345 plates. Price 12*s.* 6*d.* net.

Trial of Buck Ruxton. Edited by R. H. Blundell and G. Haswell Wilson. (London: William Hodge and Co. Ltd.) 1937. Pp. ix + 457, 16 figs. Price 10*s.* 6*d.* net.

Anatomical Society of Great Britain and Ireland

PROCEEDINGS

December 1936 *February* 1937 *May* 1937
July 1937

RECORDED BY
A. J. E. CAVE

PROCEEDINGS OF THE ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

DECEMBER 1936

The Annual Meeting of the Society was held in the Department of Anatomy and Embryology, University College, on Friday, 4 December 1936, with the President, Professor J. E. S. FRAZER, in the Chair.

Mr A. L. P. JEFFREY read a paper on the "Mechanics of derangements of the knee-joint".

Professor FRAZER commented upon the inherent difficulties of diagnosis in mechanical troubles affecting the knee-joint, and detailed the condition of the cruciate ligaments he had found in examples of genu recurvatum. Dr HUNTER criticized the premisses of certain arguments put forward in the paper. Professor WOOLLARD considered the physiological aspects of the articulation to be more important than the purely mechanical. Professor APPLETON emphasized the very real problems raised and discussed in this paper. Its author had considered the knee-joint relative to the whole body; in demonstrating the actual relative strengths of the internal and external lateral ligaments, the slackness of the cruciate ligaments in medial rotation, and the backward excursion of the lateral meniscus; his work was an advance on that of Fick, and provided a basis for an evolutionary study of this joint.

Professor T. B. JOHNSTON read a paper for Mr A. RALPH THOMPSON, entitled, "General review of congenital lesions of the urinary tract". In the discussion following, the PRESIDENT detailed the development of the infra-umbilical abdominal wall, emphasizing the fundamentally paired nature of the genital tubercle, and the included lower part of the future uro-genital sinus; failure of fusion of the halves of the tubercle left the infra-umbilical belly wall feeble, and facilitated extroversion. Sir ARTHUR KEITH criticized the application of the terms "epispadias" and "hypospadias", and remarked on the value of the series of cases presented. Professor T. B. JOHNSTON commented upon the variable nature of the cloacal membrane in embryos of similar stages of growth: he considered failure of this membrane responsible for those cases of bladder extroversion which manifest also an intestinal opening.

Dr A. J. E. CAVE read a paper on "Identification of cervical vertebrae". The PRESIDENT and Sir ARTHUR KEITH commented on the new evidence for identification submitted.

Dr A. J. E. CAVE also read a paper on "The anatomical and obstetrical significance of the sacro-sciatic notch", of which the following is an abstract.

Recent examination of some female Baganda pelves had directed attention to the significance of this notch (*a*) as an unreliable sex character, (*b*) as an

important indicator of pelvic outlet configuration. Of ten such pelves, four were from normal adults, one was immature, and five were from women dead from obstructed labour, the obstruction being due to a marked contraction of the outlet.

In contradistinction to its European counterpart the normal female Baganda pelvis is characterized by a cordate (not reniform) brim, and a narrow subpubic angle (averaging but 67° in ten specimens examined). This subpubic narrowness is easily discernible clinically, as the apex of the arch will not accommodate two fingers. The sacro-sciatic notch, however, is of good female type, i.e. is more or less rectangular, with a lengthy superior border, so that sacrum and coccyx are thrust well backwards and a sufficiently roomy pelvic outlet results. Reduction of the subpubic angle is insignificant obstetrically provided the outlet is amply capacious: the foetal head rides over the pubic bones and presents in the posterior segment of the outlet (i.e. behind a line joining the ischial tuberosities) whose adequate length is maintained by a sufficient sacral back-thrust, itself dependent upon a wide notch with long upper border. Occasionally, this subpubic narrowing is compensated for by a general increase of total pelvic antero-posterior diameter. The form and proportions of the sacro-sciatic notch constitute therefore as important a clinical factor as pubic configuration itself.

This is illustrated by the five pelves from the fatal labour cases. These specimens were remarkably andromorphic, bearing the "stigmata" described by Berry Hart (*Edinb. Med. J.* vol. XVI, 1916) so emphatically that only their pubic elements revealed their true sex. All five presented a congenital contraction of the outlet, drastically encroached upon by large ischial spines as well as by the lower sacrum and coccyx. Their pubic portions differed in no essential from the preceding (normal) group: their sacro-sciatic notches, however, were of ultra-masculine type, tending to the circular in outline, and with short upper borders having the effect of tucking the lower sacrum too closely towards the inter-ischial line. In such andromorphic pelves natural delivery is impossible; the foetal head is denied egress through the anterior outlet segment by reason of the reduced subpubic angle, and equally through the reduced posterior segment, encroached upon as it is by unyielding bone: operative interference, therefore, becomes imperative.

A comparison of these two types of negro pelvis (which can be matched in white material) demonstrates (*a*) that a reduced subpubic angle is not of serious moment provided the posterior segment of the outlet is sufficiently roomy, (*b*) that a combination of pubic narrowing with restricted posterior segment (male type of notch) is fatal, (*c*) that the form of the sacro-sciatic notch is a reliable guide to outlet configuration, a widely open notch being more important clinically than an adequate subpubic angle, as pointed out previously by Derry (*J. Obstet. Gynaec.* vol. XLII (3), 1935).

General conclusions include the following:

(1) The sole reliable criterion of sex in the innominate bone is the pubis: the symphyseal-acetabular width always exceeds the trans-acetabular.

(2) Its wide range of variation (including indeterminate and frankly male varieties) precludes the sacro-sciatic notch from use as a pelvic sex-indicator.

(3) The notch is, nevertheless, a valuable indicator of outlet configuration: and, since a wide notch can compensate for subpubic narrowing, of more practical importance in this connexion than is the pubic arch.

(4) In pelves with pubic narrowing plus the male type of notch natural delivery is impossible.

(5) The sacro-sciatic notch should receive obstetrical attention equal to, or even surpassing, that accorded the pubic arch. Its form should be determined when necessary by careful radiographic examination.

Professor BARCLAY-SMITH recalled Professor DERRY's similar conclusions on the Egyptian pelvis communicated to the Society some years ago.

Sir ARTHUR KEITH drew attention to the occurrence of a typically female type of sacro-sciatic notch in the male acromegalic.

Dr R. WALMSLEY gave a paper entitled "Structure and significance of the rete mirabile in Cetacea", of which the following is a résumé:

Observations on the retia mirabilia of Cetacea have been made on adult specimens of the finback (*Balaenoptera physalus*), the humpback (*Megaptera nodosa*) and the sperm whale (*Physeter macrocephalus*), partly on material that had been brought to the Carnegie Institute of Embryology, Baltimore, by Dr E. M. K. Geiling and partly on fresh material at Rose Harbour, Queen Charlotte Island, B.C. The general arrangement of the thoracic rete—the largest of the retia—was found to be consistent with the usual accounts of it. The rete is formed of both arteries and veins; the arterial component is much the greater. The retial arteries arise from a large dorsal thoracic artery or its segmental branches, and although they have multiple anastomoses they are of a fairly constant calibre. The thoracic rete of each side is continuous with the spinal rete by extensions which pass through the inter-vertebral foramina. There is a great disproportion in size between the spinal cord and the vertebral canal in the larger Cetacea—in an adult finback the cervical part of the spinal cord has a diameter of 1·5 cm., whereas the transverse measurement of the canal is 15 cm.—and the entire extra-dural space is occupied by the rete and the spinal veins. The caudal extension of the rete varies with the species, but the rete always extends forwards into the cranial cavity through the foramen magnum. In the region of the foramen the rete divides into right and left halves which, as they pass forward, at first deviate from the median plane and then turn medially to the sella turcica where they form a large vascular mass in which the hypophysis is embedded. Out of this mass there arise the cerebral arteries, each of which is formed by the union of several of the arteries of the rete. The cerebral venous drainage is largely effected by the venous component of the spinal and thoracic retia and terminates in the dorsal thoracic vein and its tributaries.

The significance of the retia can only be appreciated if it be remembered that the internal carotid arteries are obliterated early in foetal life and that the vertebral arteries are absent. In virtue of the great area of the arterial part of the retia as compared with the size of the trunks from which they take origin, and also on account of the numerous anastomoses, the retia must act not only as a reservoir for the blood to the central nervous system but also as a pressure-regulating mechanism for the blood vessels of that system. In addition it is to be noted that the skeleton affords the maximum amount of protection to the cerebral vessels from the large external pressures which arise during deep submergence.

Mr R. H. BURNE enquired as to the occurrence of two longitudinal spinal meningeal blood vessels in the Cetacea, such as occur in porpoises, and as to

the precise arrangement of arteries within the retia. He considered a retial blood supply to the brain not universal in cetaceans, because of the frequent persistence of their ento-carotids. Professor WOOLLARD asked about the distribution of elastic tissue in the retial vessel walls, in connexion with pressure mechanisms. Dr CAVE asked whether the retial innervation had been determined, and whether attention had been paid to the inguinal cetacean rete, accredited by some with a lymphatic connexion. Dr WALMSLEY replied that he had not found the longitudinal spinal arteries present in his material, that elastic tissue was most abundant in the walls of the proximal retial vessels, and that he had observed a liberal retial nerve-supply.

Dr E. S. DUTHIE read a communication entitled "Haemopoiesis in certain Teleostean fishes", which will be submitted to the Editorial Committee of the *Journal*.

Professor J. P. HILL congratulated the author upon his study of granulocyte development and enquired the distinction between granulocytes and lymphocytes. Professor SENIOR enquired as to the presence, around the postcardinal veins, of any *anlage* of erythrocyte formation. Dr DUTHIE replied that no such body had been noted, and (in answer to Professor HILL) that in these lower forms no sure distinction could be established between granulo- and lymphocytes: the granulocytes were of reticular origin.

FEBRUARY 1937

An ordinary Meeting of the Society was held in the Anatomy Department of St Thomas's Hospital Medical School, on Friday, 12 February 1937, the President, Professor J. E. S. FRAZER, in the Chair.

Dr C. C. TCHAPEROFF communicated a paper upon the "Radiological anatomy of the kidney and ureter", of which the following is a résumé:

The differences found in the position of the kidneys and ureters in the cadaver and living subject are contrasted by making the pelvis of the kidneys and ureters visible on radiographs by Perabrodil.

In the cadaver the kidneys appear at a higher level than in the living subject. In the cadaver the ureter, as a result of the high position of the kidneys, takes up a straight line from the pelvis of the kidney to the sacroiliac joint, so that it crosses the transverse processes of the 3rd, 4th and 5th lumbar vertebrae.

The use of intravenous Perabrodil demonstrates that the ureter is often tortuous and may lie over 1 in. medial or lateral to the tips of the transverse processes of the lumbar vertebrae. Clinically any opaque shadow lying within this area on a radiograph must be suspected as lying within the ureter till the ureter is visualized.

Under the phlorescent screen, with the kidney filled with Perabrodil, renal and ureteric systole and diastole can be seen.

Between deep inspiration and expiration the kidney descends the distance of one vertebral body, moving also slightly outwards. The ureter in compensation moves slightly inwards and becomes more tortuous.

The pelvis of the kidney shows wide variation in size, shape, tone and relation to the hilum. The long axis of the normal kidney is directed downwards

and outwards. The ureter is 1·5–7·0 mm. in diameter and is constricted at the uretero-vesical junction.

Dr TCHAPEROFF, answering an enquiry by Professor DAVIES, agreed that type of bodily habitus was definitely reflected in the varying positions of the renal pelvis as revealed by radiography.

Dr J. H. GRAY read a paper upon "The lymphatics of the stomach", which has been published in the *Journal.*

Professor WOOLLARD congratulated the author upon his successful demonstration and touched upon certain problems raised by the anatomy of the lymphatic system. Dr GRAY answered Dr CAVE that no retrograde flow in lymphatic vessels had been obtained, and that he did not regard such flow as normally possible.

Dr MATTHEW YOUNG read a paper entitled "The growth of the face", which has been published in the *Journal.*

Dr CAVE commented upon the value of Dr YOUNG's careful anthropometrical researches. Professor WOOLLARD noted that asymmetrical facial development was due to irregularities of maxillary growth.

Professor WOOLLARD read a paper upon "Continuity in peripheral nerve fibres", which has been published in the *Journal.*

The PRESIDENT remarked that, embryologically, perhaps some evidence of such continuity was to be seen in the neural loops carried forwards within advancing crests of mesoderm. Professor LE GROS CLARK asked whether the intra-vitam methylene-blue method of staining had been employed and whether this technique was successful for the demonstration of neurofibrillae. Professor WOOLLARD replied that all his preparations were made by the methylene-blue method; this was superior to silver staining, which fails to emphasize many fibres.

Dr D. J. HURRELL read a paper on "The nerve supply of bone" which has been published in the *Journal.*

Professor DAVIES congratulated the author upon his success in dealing with a very difficult problem. Professor WOOLLARD considered the intraosseous structures demonstrated to be genuine nerve fibres; the PRESIDENT expressed the same opinion, and thought the fibres of vaso-motor nature. Dr BACSICH remarked upon the greater richness of bone innervation in young animals, and the relative paucity of nerve fibres to be found in adult bone: this had been established in reptilian material. In reply Dr HURRELL emphasized the essentially preliminary nature of his communication and the technical difficulties involved in the work: his material was adult cat femur.

Dr E. S. HORNING and Mr K. C. RICHARDSON exhibited a cinema-film, in colour, demonstrating "New technique for cultivation of tissues *in vitro*". They described a new rotary apparatus, which consists of a drum containing a series of glass culture tubes rotated by a motor, a modification of the apparatus described by Gey (*Amer. J. Cancer*, vol. XVII (1933), p. 752). The advantages of this method of tissue culture are to be found in the continual circulation of the fluid medium and the simplification of the various manipulations necessary for cultivating a large number of explants for prolonged periods. Larger explants can be maintained in a healthy condition without the central necrosis so

common in the stagnant medium of flask and hanging-drop cultures. In addition, the film demonstrated the routine procedures for preparing fowl plasma, serum, and chick embryonic extract, together with the technique of setting up the cultures, changing the medium, and adjusting the hydrogen-ion concentration.

The PRESIDENT congratulated the authors upon the ingenuity of their technique and upon the excellence of their very successful demonstration. Dr HONOR FELL tendered congratulations upon the authors' modification of the tube technique, and upon the high standard of photography in the film. Professor J. P. HILL observed that the exhibition of this particular technique in coloured cinematograph form was the first of its kind in this country; the methods displayed were capable of fruitful employment in tackling the problems of experimental embryology.

Mr J. WHILLIS read a paper upon the "Development of synovial cavities", whereof the following is an extract:

A section through a developing diarthrosis in the foot of a 30 mm. human embryo reveals the two main chondrifying elements separated by a disk of relatively undifferentiated cells: these cells have a general arc arrangement, save towards the future articular surfaces, where they assume a flattened disposition, whilst at the disk's periphery, where there is a change from the arc to longitudinal disposition, the cellular elements are more loosely packed.

At a slightly later growth stage there is manifest a still further flattening of the disk cells, a diminution in relative thickness of the disk, and the appearance of a cavity in the circumferential portion of the disk, whose cells are beginning to assume positions parallel to the boundaries of the space.

Further growth stages reveal a continued diminution of the disk—which is ultimately reduced to a single cell layer—and a progressive extension of the circumferential disk cavity, which becomes finally lined by flattened cells.

At an advanced foetal age, joint sections show the skeletal elements to be mutually united by primitive cartilage; the cartilage at the future articular surfaces is shown by special staining to be fibrillated; a well-marked circumferential cavity is apparent, with some partial remains of the disk.

The successive histological pictures are believed to support the assumption that the disk undergoes compression (the cartilage growing much more rapidly than the surrounding tissues) because (*a*) there is flattening of the cells arranged in arcs, (*b*) there is progressive diminution in disk thickness, and (*c*) during compression of a disk of deformable, laminated material the stresses arising would account mechanically for the circumferential site of the resultant cavity. If, then, "compression" be a factor in disk abolition, evidence should be forthcoming of pressure relief upon the intervening mesenchyme in those situations wherein disks persist: sections through the developing wrist-joint seem to provide such evidence. In early stages, both radial and ulnar styloids articulate with the carpalia (cf. the condition in the wrist of the macaque monkey), mesenchyme interposes between the carpus and the lower ends of the two long bones. A later section shows a "withdrawal" of the ulnar head from contact with the carpus, and the mesenchyme forming the intra-articular disk and medial lateral ligament. At the same time the radio-carpal articulation has progressed almost to the stage of union of skeletal elements by primitive cartilage.

The conclusions reached from this preliminary study are (1) that the presence of a mesenchymal cell disk is essential in the formation of a synovial joint, (2) there is a gradual disappearance of this disk either by incorporation in the extremities of the skeletal elements, or by a compression atrophy, and that where compression is absent the disk persists, (3) that the joint cavity first appears circumferentially, as a result of stresses due to compression, (4) that the bone elements are united, after disk disappearance, by primitive cartilage, the union being easily broken by rough handling or by shrinkage in preparation. This union persists up to the time of foetal movements (in man), its breakdown being due to either (*a*) the foetal movements themselves or (*b*) the slowing of cartilage growth by beginning calcification, i.e. by a relative shrinkage.

In the subsequent discussion the PRESIDENT recalled former similar studies by Jenkins, and expressed some personal uncertainty concerning the phenomenon of cartilaginous structures undergoing pressure changes. Dr HONOR FELL had studied similar material: in tissue-cultivation experiments she had encountered partial separation of articular surfaces in the explanted limb-bud, but had never observed the development *in vitro* of any real joint cavity. Whether the failure of cavity formation was due to poor nutrition or to absence of the normal foetal movements she could not decide. Certainly, in developing chick tissue, cartilage continuity across the future joint cavity was plainly manifest by all histological criteria.

Professor LE GROS CLARK enquired as to the manner whereby the secondary fusion of cartilaginous skeletal elements was subsequently reversed in the final stages of joint development.

Professor HARRIS suggested the employment of various stains and fixation methods to distinguish growth changes from the effects of movement by emphasizing mitotic figures. He had never observed personally the secondary fusion and absorption of skeletal elements described by Mr WHILLIS.

Dr J. D. BOYD communicated the following papers: (*a*) "Nerve supply of ductus arteriosus in the rabbit."

The ductus arteriosus of the rabbit foetus, near full term, possesses a rich innervation derived partly from the left aortic arch ("depressor") nerve and partly from the left vagus nerve. The fibres reaching the ductus are of two sorts: thick fibres which terminate chiefly in the adventitia but partly in the media of the vessel wall and thin fibres which are mainly in the media. The thick fibres terminate in a manner very similar to the pressor receptor endings found in the carotid sinus, in the aorta and in the right subclavian artery. The thin fibres terminate in relation to the muscle fibres of the media of the ductus. In the ligamentum arteriosum of the adult rabbit neither type of nerve fibre was found. No pressor receptor endings were found in the portions of the pulmonary arteries outside of the lungs in either foetal or adult rabbits.

It is suggested that the presence of pressor receptors on that portion of the right sixth branchial arch artery which becomes the ductus arteriosus increases the evidence in favour of the view that the persisting portions of the embryonic branchial arch vessels are sites of election for the development of specialized pressor-receptor mechanisms. It is further suggested that the presence of a rich nerve supply to the ductus arteriosus may be correlated with those features of its histological structure which are the anatomical basis for its

sudden closure at birth. The recent work of Sir Joseph Barcroft on the foetal circulation has shown that the Sabatier doctrine which implies this sudden closure is supported by physiological evidence. A study of the wall of the ductus shows that there is in this portion of the arterial system a marked diminution in the collagenous and elastic tissue fibrils and an increase in the number of the muscular fibres in the media. The initiation of the contraction, at birth, of this thickened, sphincter-like media is probably conditioned by the pressure changes in the ductus when the blood is suddenly re-routed and it is with this part of the process that the specialized nerve supply may be concerned. No suggestion can be made as to the manner in which the contraction is maintained during the relatively long period (3–4 weeks) which elapses before there is anatomical obliteration of the lumen of the ductus.

(*b*) "Proprioceptive innervation of mammalian tongue." Tarkhan (*Z. ges. Anat.* Bd. CV, 1936) has described proprioceptive fibres passing in the hypoglossal nerve of the rabbit, and he states that the cell bodies of these fibres form a ganglion on the peripheral part of the nerve. He also reaffirms Langworthy's description of muscle spindles in the tongue of the rabbit. Barron (*Anat. Rec.* vol. LXVI, 1936), on the other hand, was unable to record impulse discharges from the hypoglossal nerve of the cat or rabbit when the tongue was stretched or distorted. He did, however, obtain such discharges from the lingual nerve, but the discharges, when single-fibre preparations of the nerve were studied, did not have as high an initial frequency as that described by Matthews for muscle-spindles and the end-organs producing the discharges adapted more rapidly.

A study of complete serial sections through the heads of full-time rabbit foetuses, cut in the transverse, sagittal and coronal planes, and stained with de Castro's modification of the Ramon y Cajal technique, did not show any evidence for the presence of hypoglossal sensory cells such as are described by Tarkhan. No trace of a Froriep's ganglion (stated by Langworthy to be commonly present in the rabbit) was found. Further, no histological evidence was found for intra-medullary fibres of the hypoglossal nerve having direct connexions with any cell groups other than the nucleus of Stilling itself.

In full-time foetal and adult rabbit tongues (stained by the de Castro, Bielschowsky and methylene blue techniques) no muscle-spindles could be found in the intrinsic musculature and none were found in the portions of the extrinsic muscles which were retained in the sections. There is a rich arborization of nerve terminations in the connective tissue interstices of the tongue, but in the absence of degeneration experiments no statement can be made as to their nature and origin. Nothing resembling a true muscle-spindle with perilymphatic space, epilemmal ending and intra-fusal fibres was seen, however, so that the investigation falls into line with the results of Sherrington, Batten, Baum, Cippolone and Boeke, all of whom have denied that muscle-spindles are present in the intrinsic tongue musculature.

The PRESIDENT enquired as to the fate of Froriep's ganglion: he considered the trigeminal nerve a more hopeful route than the first cervical, for proprioceptive impulses. Professor WOOLLARD referred to Langworthy's work, to the proprioceptive impulses from the facial area and to the variation in different species of the proprioceptive nerve endings in the extra-ocular muscles.

Professor LE GROS CLARK referred to experimental evidence for proprio-

ceptive impulses in the hypoglossal nerve, pupillo-ocular reflex being the criterion taken. Though Dr CARLETON had been unable to demonstrate proprioceptive fibres in the proximal hypoglossal, he considered their presence certain.

Professor HARRIS commented upon the absence of any ganglion cells in the hypoglossal nerve of the pig embryo. He considered the tongue not to require a proprioceptive innervation akin to that of the truly postural muscles, inasmuch as its spatial relationships were relatively unimportant.

Dr J. FORBES read a paper on "*Thoracopoagus monosymmetros* with congenital club hand", which will be submitted for publication in the *Journal*.

The PRESIDENT remarked upon the rarity of double caecum and appendix, such as this specimen showed. Professor NICHOLSON enquired whether mechanical pressure alone might not explain the club hand. Dr BRANDT had observed absence of forearm bones in *Amblyostoma*, the hand articulating with the humerus. Dr HARPER thought the digit termed "index" might morphologically represent some other digit. In reply, Dr FORBES discounted mechanical pressure in favour of a primary tissue defect as the underlying cause of the malformations present in his specimen.

Dr R. WHEELER HAINES read a paper upon "The evolution of epiphyses" which will be submitted for publication in the *Journal*.

The PRESIDENT, Professor APPLETON, Professor HARRIS and Dr CAVE discussed various points raised in this communication.

MAY 1937

An ordinary Meeting of the Society was held in the Anatomy Department of Guy's Hospital Medical School on Friday, 21 May, with Professor T. B. JOHNSTON in the Chair.

Mr S. WILSON CHARLES read a paper on "Development of the mandible".

Professor HARRIS remarked that the site of the developing first permanent molar was accurately indicated, in mammalian mandibles, by the apex of the triangular cartilaginous mass occupying the ascending ramus: he suggested sections across this mass in future investigations.

Professor DAVIES asked concerning the connexion between the gubernaculum and the mode of attachment of the developing first and second permanent molars.

In reply, the author emphasized the relation of the gubernaculum to the anterior teeth only: it had no connexion with the molars.

Professor G. W. NICHOLSON described "A disorganized human embryo".

Professor LUCAS KEENE stressed the general absence of blood vessels in similarly malformed specimens. Professor WOOLLARD enquired as to the embryonic nutritional system in the absence of vascular channels. Professor NICHOLSON replied that the embryo survived much in the manner of an experimental transplant.

Professor T. B. JOHNSTON demonstrated "A human embryo with early development of primitive streak", an account of which will be published in the *Journal*.

Professor HILL tendered congratulations upon the successful outcome of a very difficult task of reconstruction. He considered the curvature of the embryonal shield abnormal, Hensen's node to be well developed, and a head process to be almost certainly present. The relative shortness of the primitive streak was well shown by the specimen, whose cloacal-membrane region, unlike the simpler corresponding region of lower Mammalia, was most difficult of interpretation. Though he doubted whether the exocoelom actually invaded the embryonic area, he agreed in the main with Professor JOHNSTON's reading of the evidence presented.

Dr ODGERS commented upon the different structural characters of the dorsal and ventral walls of the yolk-sac. In reply, Professor JOHNSTON expressed his indebtedness to Professors HILL and FLORIAN for their co-operation in the study of this embryo, and, at Professor WOOLLARD's request, explained again certain of his diagrams. He still thought the specimen showed a precocious intra-embryonic coelom.

Mr A. L. P. JEFFERY communicated "A note on the popliteus muscle" supporting his remarks by "Models showing mechanical stresses".

Dr MACCONAILL regarded the popliteus as a Winslowian "articular muscle"; in its functional relations to the mechanism and ligaments of the knee-joint it was comparable to the obturator externus in connexion with the hip-joint and the ischio-femoral ligament. It assisted in the rolling movement responsible for the correct disposition of synovial fluid within the knee-joint. Regarding the cruciate ligaments, he pointed out that in extension of the joint (before the beginning of terminal rotation or locking), both cruciates and both collateral ligaments were tense; at the commencement of terminal rotation (the screw-home movement), the collateral and anterior cruciate ligaments alone remained tense, and the posterior cruciate slackened off. He regarded ligaments as synergic to the peri-articular muscles.

Dr STEWART described certain experimental findings on the hind-limb muscles of rodents: after section of the gastrocnemius, etc., the popliteus remained as a powerful rotator of the foot.

Dr WHEELER HAINES commented on the role of the popliteus as part of a rotatory mechanism correlating the mechanisms of foot and knee in certain primitive forms. In reptiles the muscle has purely tibio-fibular (and no femoral) attachments, and is a rotator of the foot. In man and mammals the muscle has deserted its old (fibular) for a new (femoral) attachment; this change has necessitated the creation of a new ligament (the fibular collateral) from, probably, the substance of the peroneus longus.

Professor APPLETON and Dr CAVE also spoke, and the author replied.

Professor MAINLAND communicated a paper on "Cell-size with special reference to the human lymphocyte", which will be submitted for publication in the *Journal*.

Dr MACCONAILL asked whether there was any correspondence of the cell-size seen in frozen-section material (i.e. cells in the aggregate) and that observed in film preparations.

Dr YOFFEY emphasized the importance of cell-size in the study of the life cycle of lymphocytes. He had observed that lymphocytes from the blood stream exceeded in dimensions those taken from the lymphatics; if measure-

ments were reliable it appeared that the lymphocytes actually increased in size whilst circulating in the blood stream. In smear preparations also lymphocytes appeared to be bigger than in fluid preparations. He thought the different osmotic pressures of blood and lymph may very materially influence the size of lymphocytes.

Professor MAINLAND, in reply, said he could not answer definitely Dr MACCONAILL'S query. He agreed that osmotic pressure was certainly a most important factor in cell-size, and expressed himself as dissatisfied with Weissmann's observations and their interpretation.

Dr J. K. WESTON read a paper entitled "Comments on the vestibular nuclei". The comparative anatomy of the vestibular nuclei of reptilian, avian and mammalian forms was considered. The nucleus tangentialis was observed to demonstrate the greatest variability of any of the vestibular nuclei: its degree of development paralleled closely the degree to which side-to-side trunk and tail movements were concerned in the methods of locomotion of the various animals studied.

Professor WOOLLARD congratulated the author upon his excellent studies and upon his clear demonstration of the association of functional activity with cerebral morphology.

Dr CAVE commented upon the avian tangential nucleus.

Mr A. F. HUGHES read papers upon "The area vasculosa of the embryo chick" and the "Influence of circulation upon the diameter of vessels", whereof the matter will be published in the *Journal*.

In discussing the early formation of embryonic vessels and the volume of the developing blood stream Professor WOOLLARD gave a résumé of his personal researches in this connexion, illustrated by slides.

Dr J. H. GRAY read a paper on "Regeneration of lymphatics in human tissues". Regeneration and proliferation of lymphatic vessels of the human skin were described as found to occur in three types of material:

(1) Large skin grafts in which the whole skin had been transplanted as a vascularized flap from the anterior abdominal wall to the volar surface of the arm and forearm. These skin flaps had been grafted in such a way that there could have been no accurate alignment of the lymphatic trunks already in the flap with those in the arm skin around the edges of the flap.

(2) A tubed pedicle made from the skin of the iliac region of the anterior abdominal wall by the usual method of making two straight parallel incisions followed by turning under and suturing together the two edges of the strip of skin thus freed from the deep fascia. The length of the pedicle that was available was 6·5 cm., and this portion was the lower and medial end of the original pedicle which lay parallel to the inguinal ligament and was about 15–18 cm. in total length. The pedicle had been left untouched on the abdomen for 3 weeks before the specimen was removed.

(3) Skin of the leg distal to an epithelioma which was circumferential in growth about the ankle, and had involved the skin of the leg in front and at the sides of the ankle leaving only a narrow wick of normal skin posteriorly to drain the lymph from the whole foot.

The lymphatics in these cases were injected with thorotrast by the method of Woollard and Gray communicated to the Anatomical Society in 1935 (*J. Anat., Lond.*, vol. LXX, p. 195).

The work of other investigators has shown that human subcutaneous lymphatic trunks have a very constant topography in the different regions of the body, a constancy in position, number, and particularly in direction, for each region. Control work by the author on normal skin has confirmed this fact.

It is considered that the important problem of the conditions and stimuli necessary for lymphatic regeneration and proliferation should be distinguished from the question of the direction and valvular orientation developed later in the final new lymphatic trunks. From common clinical experience and from the experiments of F. L. Reichert (*Arch. Surg. Chicago*, 1926, vol. XIII, p. 871) and others it is well known that lymphatics will regenerate across ordinary sutured incisions, and apart from the third type of material used in the present work there is nothing to add to our understanding of the major problem in lymphatic proliferation.

In regard to the direction and orientation of valves in newly formed lymphatics it would appear from the first type of material (the large skin grafts) that regeneration and remodelling of the trunks tend to follow, in direction at least, the lines of the normal arrangement for the surrounding region. In the transplanted abdominal skin new lymphatic trunks were developed having a course identical with that of the vessels in normal arm skin at the site of transplantation.

The results obtained in the second and third types of material were different from those found in the large skin grafts, and it is suggested that other conditions or forces were at work in the tubed pedicle and the epithelioma cases that were not present in the simple large grafts. Thus in the tubed pedicle the larger lymphatics run along the tube in a direction away from the inguinal region to which they must ultimately go on emerging from the upper and lateral end of the pedicle. So that it can be inferred that in the course of 3 weeks new lymphatic trunks were formed, either from existing anastomoses or as entirely new products, running for some 15 cm. along the pedicle from its lower and medial end to its upper and lateral end in a direction opposite to that which would have been the shortest way to the inguinal glands. In the case of the circumferential epithelioma lymphatic trunks in the skin just behind the malleoli ran medially into the area of the narrow wick of normal skin instead of diverging laterally to join one or other of the two groups of trunks ascending near the long and the short saphenous veins, as is always found in a normal leg. It is suggested that in the latter two types of material investigated other factors at present undetermined have modified the simple rule that would appear to hold from the cases of transplanted skin flaps.

In regard to the factors causing proliferation of lymphatic vessels, the third type of material, the epithelioma cases, showed that in the enlarged dermal papillae at the margins of the malignant growth there had developed considerable plexuses of lymphatic capillaries. In this case at least liberation of the lymphatic endothelium by incisions was not necessary to cause proliferation of the vessels.

Work is in progress towards further analysis of the factors involved in lymphatic regeneration and in proliferation of lymphatic vessels.

Drs J. S. BAXTER and J. D. BOYD communicated a joint paper upon "Abnormal development of brain in a pig embryo", which will be submitted for publication in the *Journal*. Professor HARRIS complimented the authors

upon their careful avoidance of the term "anencephaly", which, on the grounds of the absence of the three mid-brain nerves (III, IV, VI) he considered inapplicable to their specimen. The condition present was rather one of "split brain".

JULY 1937

The Summer (and Jubilee) Meeting of the Society was held in the Department of Human Anatomy, University Museum, Oxford, on Friday, 2 July, and Saturday, 3 July 1937, with the Senior Vice-President (Professor H. H. WOOLLARD) in the Chair.

Messrs W. E. LE GROS CLARK, T. MCKEOWN and S. ZUCKERMAN read a paper on "Visual mechanisms in gonadal stimulation". Previous studies by other workers suggest that activities of the gonads in the ferret during the anoestrous season by exposure to artificial light is dependent upon stimulation of the retina. In the present paper the authors record a series of experiments designed to determine the neural mechanisms in the brain which are involved in this process of gonadal stimulation. Under nembutal anaesthesia, experimental lesions on the brain in a number of female ferrets were made in November and December of last year (1936). The lesions were as follows: (1) ablation of the visual cortex in both hemispheres, (2) destruction of both superior colliculi, (3) bilateral section of the optic tracts at the ventral border of the lateral geniculate bodies, cortex and mid-brain, and (4) retrobulbar section of both optic nerves. All animals were transferred within a few days of operation to a room arranged for exposure to prolonged illumination. They were placed before a large window in separate cages. Four 100 W. bulbs were fixed at a distance of 2 ft. from the front of the cages. The lamps were controlled by a time switch, arranged so that artificial illumination should begin at 4.30 p.m. and end at 11 p.m. The animals were examined twice a week, and as soon as swelling of the vulva indicated that the ferrets were in full heat they were mated with normal males who had also been exposed to artificial light to ensure their activity before the normal breeding season. After 3–4 days the females were killed. Sections were prepared of the uterus, and serial sections of the ovaries were made to determine whether ovulation had occurred. Normal control animals were also used, some being kept under normal conditions of daylight, and some being exposed also to artificial light.

The results of the experiments are recorded in the accompanying table. As the table indicates, normal unoperated animals exposed to artificial illumination came into heat after periods of from 50 to 70 days of light stimulation. Those animals in which the lesions had involved the visual cortex, the superior colliculi or the optic tracts came into heat approximately within the same periods (although there is some indication of a slight delay in the response of the animals with the collicular lesion). One of two animals exposed to artificial light after retrobulbar section of the optic nerve did not come into heat until after a period of 142 days (i.e. on 24 April, by which time many control ferrets kept under normal laboratory conditions were also in heat). The other animal in which the optic nerves were sectioned had not come into heat at the time of writing (8 July). Serial sections through the brain showed that in ferrets F. 14 and F. 15, the superior colliculi had been completely severed from the rest of the mid-brain, while the pretectal nucleus and its connexion with the mesencephalic fibres of the optic tract remained intact. In the case of the

optic tract lesions, serial sections showed that in both these experiments the whole tract had been completely severed on one side, while on the other side the lesion was incomplete, a small strand of fibres passing to the mid-brain having been left intact. The experiments, so far as they go, indicate that the visual cortex and the superior colliculi play no essential part in the response of the gonads to visual stimuli. It remains possible that this response is dependent on impulses which pass either to the pretectal nucleus by mesencephalic fibres of the optic tract, or to the region of the subthalamus by way of accessory optic tracts which have been described as terminating (*a*) in the region of the subthalamic nucleus (anterior accessory optic tract), or (*b*) in the region of the nucleus opticus tegmenti (posterior accessory optic tract or tractus peduncularis transversus). These experiments are being repeated and extended during the coming winter.

Table I

Operation	Animal	In light	Full heat	No. of days	Ovulation
Normals	F. 18	Dec. 3	Feb. 15	74	+
	F. 19	,, 3	,, 5	64	−
	F. 24	,, 17	,, 5	50	+
Visual cortex	F. 23	,, 11	,, 11	62	+
Optic tract	F. 20	,, 7	,, 22	77	+
	F. 22	,, 11	,, 11	62	+
Colliculi	F. 14	,, 3	Mar. 8	95	+
	F. 15	,, 3	Feb. 25	84	+
Optic nerves	F. 16	,, 3	Apr. 24	142	−
	F. 25	,, 19	?	?	
		No light			
Normals	F. 28	—	April 4		
	F. 29	—	June 14		
	F. 30, etc.	—	Before May 3		

Drs T. McKeown and S. Zuckerman read a paper upon "The stimulation to ovulation".

The ferret is an animal that is believed to ovulate only as a result of copulation. Experiments were therefore performed in order to determine the importance of nervous stimulation of the cervix uteri in the process of ovulation. The body of the uterus, the cervix, and upper vagina of two females in full heat were denervated by means of the injection into the parametrium of a solution of phenol. The two animals were subsequently mated. Ovulation had occurred in one. In view of this result, six other females in full heat were used to determine whether vaginal penetration by the male is essential if mating is to result in ovulation. These animals were mated in such a way that penetration was impossible. Ovulation occurred in three. It is therefore concluded that the exteroceptive stimulus necessary for ovulation in this species is less specific than is commonly supposed.

Drs T. McKeown and S. Zuckerman read a paper on "Factors involved in luteinization".

The influence of the corpora lutea on the suppression of oestrus in pseudo-pregnant, pregnant, and lactating rats was studied by the extirpation method, the corpora lutea being destroyed by means of a thermocautery needle. The results confirm the view that in pseudo-pregnancy and early pregnancy the inhibition of oestrus is immediately dependent on the presence of the corpora

lutea. On the other hand, they show that the suppression of oestrus in the second half of pregnancy is determined by the placenta, since destruction of corpora during this reproductive phase is not followed by the appearance of oestrus except in unusual cases in which abortion also follows the operation. The corpora lutea are however essential for the well-being of the foetuses, many of which die after the operation. Destruction of the corpora lutea of lactation is also not succeeded by oestrus unless the young are removed, and it is concluded that in this reproductive phase follicular inhibition is an effect of suckling.

Dr P. N. B. Odgers read a paper on the "Development of the pars membranacea septi in the human heart". This was based on observations in nine embryos between 11·2 and 17·5 mm. c.r. in length, in five of which reconstruction models of this region of the heart were made. He showed that the attachment of the left bulbar ridge to the interventricular septum may be said to divide the original interventricular foramen into two parts. That part of it which is above and dorsal to this attachment remains patent as the aortic channel; that part of it which is below and ventral is filled up by the active proliferation of the right tubercles of the auriculo-ventricular cushions to form the lower (ventral) half of the pars membranacea septi, the rest of the latter being developed from the a.-v. cushions themselves.

The proximal bulbar septum is finally attached by its inferior border anteriorly to the interventricular septum and posteriorly to the right margin of the a.-v. cushions and between the two to the cushion tissue, which has proliferated from the latter, to fill up the interventricular foramen, the proliferating tissue connecting the two bulbar ridges, and so completing the bulbar septum inferiorly. While the bulbar septum separates the aortic and pulmonary channels, it takes no part in the occlusion of the interventricular foramen.

Professor Waterston complimented the speaker upon the presentation of his interesting account, but considered the critical examination of a much wider range of material necessary before drawing final conclusions concerning developmental sequences.

Professor Davies thought that in certain sections displayed, there was already evidence of the developing bundle of His.

Dr G. R. de Beer read a paper upon the "Development of the diaphragm in *Pipistrellus*". The formation of the diaphragm is due, not only to the appearance of new partitioning membranes, as a result of local proliferation, but also to extensive transformation of shape of existing structures. Thus, the pleuro-peritoneal membranes arise as partitions in the sagittal plane. This present communication is merely preliminary, since the main problem to be attacked is the question of the provenance of the musculature of the diaphragm.

Professor D. Mainland read a paper upon "A comparison of percussion and radiology in locating the borders of the heart". This investigation was undertaken because percussion is used in the Dalhousie University Anatomy Department as a method of "dissecting" the living body. It was concerned with the practical (clinical) conception of percussion error—the difference between percussion and teleradiographic results. An experienced physician percussed on 110 male university students the deep cardiac dullness and

superior mediastinal vessels, and fastened brass wires on the students' chests to indicate his findings in intercostal spaces L 1–5 and R 4. In radiographic films made, with a 6 ft. tube-film distance, immediately after percussion, the distances between the shadows of the wires and the borders of the heart (or vessel) shadows were measured. All results were analysed biometrically. Percussion and radiographing were always done with the students in the mid-phase of respiration.

Mean errors and variations were obtained from about ninety students. In the erect position the mean error was about $1\frac{1}{2}$ mm. in space L 1, and about $\frac{1}{2}$ mm. in space L 2, neither mean being significantly different from zero. In spaces L 3, 4 and 5 the percussion wire shadow was on the average about 6 mm. lateral to the cardiac shadow (a statistically significant error, but perhaps of no great practical importance). In space R 4 the percussion wire shadow was on the average about 13 mm. medial to the edge of the cardiac shadow. In all students the percussion and filming were repeated in the recumbent position, and the mean errors were of similar type to those in erect position, but numerically somewhat different.

The possible error in the individual student or patient is much more important than the mean error. In space L 4 of an erect subject the shadow of the percussion marker might in the film be anywhere between $2\frac{1}{2}$ cm. lateral and 1 cm. medial to the edge of the cardiac shadow; occasionally beyond these limits. This variation had not been produced to any important extent by respiratory movement of the chest, and consequently of the wires, between percussion and filming. Change in transverse diameter of the heart, apparently due largely to diaphragm movement, was important in producing percussion error, and this factor would remain, however good the percussion method.

Knowledge of the stature, weight and chest dimensions of an individual would not enable a physician to prophesy the percussion more closely than if no allowance were made for these factors. (Females with thick subcutaneous fat and large mammary glands were not investigated; the subjects chosen were purposely those which would allow the physician to work under favourable conditions.) Percussion repeated on the same individuals showed a minor degree of constancy of the error, but the variation in error in any one individual was for practical purposes the same as between different individuals.

The error of any one observer is not necessarily the same as that of another, and each should test his own. Duplicate films on ten subjects sufficed to show the difference in accuracy of two physicians; but for reliable estimates of mean and variation at least about ninety subjects are necessary. To avoid the expense of films it is suggested that much information could be obtained by a simple fluoroscopic screen, if adequate attention were paid to the possible obliquity of the X-rays.

Professor WOOLLARD, Dr GRAY and Dr BARCLAY commented upon this communication.

Dr J. M. YOFFEY read a paper on "Enterectomy and the blood lymphocytes". He presented the results of a series of experiments devised to test the view of Bunting and Huston that lymphocytes are eliminated from the blood by passing through the mucous membrane of the intestine into its lumen. In previous experiments it had been shown that if a dog were anaesthetized by intravenous chloralose there was a marked fall in the blood lymphocytes. By

measuring the thoracic duct lymphocyte-output one could show that while in some cases the lymphocyte output fell, in most it either remained fairly constant or showed a rise. Removal of most of the small intestine seemed to prevent the fall of the blood lymphocytes in many cases, although in some experiments even after enterectomy the blood lymphocytes fell. The tentative conclusion was drawn that while undoubtedly the intestine does play a part in the elimination of lymphocytes from the blood, they can also disappear from the blood in large numbers in the absence of the greater part of the intestine.

The CHAIRMAN commented upon the great importance of this research upon the vascular tissues, from both the anatomical and clinical aspects: he congratulated Dr YOFFEY upon the industry and persistence wherewith he had prosecuted his enquiries.

Dr J. S. BAXTER read a paper upon "Vaginal development in Mammalia". Study of a number of mammalian forms has indicated that in many the vagina is developmentally compound, that is, structures other than the Müllerian ducts enter into the formation of this organ, especially at its caudal end. The clear picture presented by *Didelphys virginiana* (where the caudal part of each lateral vaginal canal is formed from the urogenital sinus), urged the study of a eutherian form where the lower part of the vagina shows duplicity. Such a form is *Bradypus griseus* (the three-toed sloth), in which the lower one-third of the vagina is in the form of two separate canals.

It has been found that the duplicity of the lower end of the vagina in this form represents solely a persistence of the primitive, separate, caudal segments of the Müllerian ducts in the genital cord. Neither the Wolffian ducts nor the urogenital sinus contributes in any degree to the formation of the vagina in this form.

This investigation therefore emphasizes the fact that vaginal development in the Mammalia is a very variable phenomenon. Whether or not the developmental process in *Bradypus* is to be regarded as primitive is very doubtful.

Dr HUNTER disagreed with Dr BAXTER on certain points: he defined the vagina as extending from hymen to cervix, and as contributing an embryological entity. Dr ZUCKERMAN stressed the importance of the endocrinological viewpoint and the need for consideration of tissue reaction to hormonal stimulation in elucidating embryological problems. The varied responses of the vaginal mucosa to oestrin injection showed that in certain animals the vagina was definitely of Müllerian origin, but that in others it derived from the urogenital sinus: the hymen was in no sense an anatomical limit to the vaginal tract.

Dr AMOROSO asked whether, in the developing rabbit vagina, any recognizable histological difference of epithelium distinguished the Müllerian and urogenital-sinus portions: in reply, it was stated that no such epithelial distinction could be detected.

Professors T. THOMPSON FLYNN and J. P. HILL read a paper upon "Maturation, fertilization and early cleavage in the Monotreme".

The authors exhibited a series of lantern slides illustrating the formation of the 1st and 2nd polar bodies, the approximation and fusion of the male and female pronuclei and the early stages in the meroblastic cleavage of the egg, including stages with 4, 8, 16 and 32 cell areas in the blastodisk.

Attention was called to the assumption by the germinal disk, prior to

cleavage, of a bilateral symmetry, characterized by the oval form of the disk, the position of the two polar bodies at one of its ends, and the greater abundance of fine, superficially situated yolk spheres at the opposite end. The first cleavage plane is transverse to the long axis of the disk but is situated nearer the more yolk-rich end, so that the two resulting cell areas are of unequal size, the smaller tending to be richer in yolk spheres than the larger. They confirmed the existence round the ripe ovarian ovum of the layer of material, originally described by Caldwell under the name of the "proalbumen". They showed that this material, situated between the follicular epithelium and the zona, is secreted by the follicular cells, and they suggested that it is the homologue of the liquor folliculi of the Graafian follicle of other mammals.

Dr J. K. Weston read a paper upon "Fibre connexions of the vestibular nuclei". A common pattern of vestibular connexions is apparent in reptiles, birds and mammals. These include root fibres to all the homolateral vestibular nuclei, to the cerebellum, and possibly to contralateral vestibular nuclei, as well as secondary connexions into both homo- and contralateral medial longitudinal fasciculi, into the reticular grey matter of either side, and interconnexions with spinal cord and cerebellum. In the smaller, more active lizards, in birds and in mammals a more direct and specialized homolateral ventrolateral vestibulo-spinal tract is present.

Professor Le Gros Clark, remarking upon the excellence of the study presented, asked whether the histological findings had as yet been subjected to confirmation by the criteria of degeneration experiments.

Professor Lucas Keene enquired whether any communication had been noted between the vestibular nuclei and the posterior commissure. A negative reply was given to both questions.

Dr Una Fielding read a paper on the "Thalamus of *Notoryctes*", which described the nuclear pattern of the thalamus in the marsupial mole *Notoryctes typhlops* and showed drawings from sagittal and transverse sections of this brain.

The dorsal part of the diencephalon (pars dorsalis diencephali of Le Gros Clark) in this creature shows the mammalian pattern in its simplest form. Not only does the lowly phylogenetic position of the creature make its brain valuable to the comparative neurologist, but the fact that it is totally blind also helps to lay bare the fundamental form.

The rarity of the creature and the difficulty in reaching its habitat (the deserts of central and north-western Australia) make well-fixed material almost impossible to obtain. That which has been used in this work comes from several sources, and there is no series which is both well fixed and complete. However, from the five imperfect series now available it has been possible, by making projection drawings of a great number of sections and restaining many slides by several methods, to decipher the nuclear pattern and most of the fibre paths.

The thalamus proper consists, on each side of the mid-line, of an ovoid mass of large- and medium-sized cells, across which in its caudal three-fourths runs a transverse lamina of small cells dividing the mass into upper and lower levels. At the mid-line this transverse lamina of small cells fuses with its fellow of the opposite side. Here, in the massa intermedia, it is crossed at right angles by a vertically running layer of fibres and small cells forming the

medial edge of each thalamic mass. This is the diencephalic periventricular system, interconnecting the pars ventralis with the pars dorsalis diencephali. So there appears in transverse sections of the thalamus proper except in its cranial fourth a cruciform arrangement of small cells separating the large and medium-sized cells into right and left upper groups (mainly nucleus dorsomedialis) and right and left lower groups (mainly ventral nucleus) of each side. The dorsal part of the lateral geniculate body is not distinguishable, but the pars ventralis is present.

The ventral nucleus at its caudal end passes over gradually into the medial geniculate body.

The cranial fourth of the thalamus proper, where there is no transverse lamina of small cells, but where the periventricular system is well marked, is occupied by the anterior nuclear group. This area does not show great differentiation, but a nucleus anterior medialis and a nucleus anterior ventralis can be distinguished. There is no nucleus anterior dorsalis in this brain. Caudally the anterior nuclear elements pass over gradually into the ventral nucleus below the transverse lamina of small cells and into the nucleus dorsomedialis above it.

The large lateral habenular nucleus shows interesting features both in its cell differentiation and its fibre connexions.

A full account of the thalamus of *Notoryctes* will be submitted for publication in the *Journal of Anatomy*.

Professor Le Gros Clark congratulated the author upon the investigation of so morphologically instructive a brain.

Professor E. S. Goodrich read a paper on "The spinal nerves of the Myxinoidea". In the formation of the spinal nerve of the Gnathostomata the mainly sensory ganglionated dorsal root joins the motor ventral root to form the mixed spinal nerve. This nerve passes outwards posteriorly to the myomere supplied by its ventral root. Moreover, between the segmental myomeres intersegmental arteries from the dorsal aorta run up dorsally, passing posteriorly to each spinal nerve. Segmental nerves and intersegmental arteries, therefore, alternate. These relations are constant from fish to man.

In the Cyclostomata, a more primitive group of vertebrates, the conditions are different. In the lampreys or Petromyzontia, the ventral root remains independent and does not join the dorsal root to form a mixed spinal nerve. It passes directly to its myomere, while the dorsal root runs outwards posteriorly to this myomere. The closely allied hag-fish or Myxinoidea, however, have mixed nerves, since the dorsal root joins the ventral root anterior to it, as in fishes. The condition in Petromyzontia is almost certainly primitive, for in *Amphioxus* also the dorsal and ventral roots remain separate. There is reason to believe, as suggested by Koltzoff, that the mixed nerve has been acquired in the Myxinoidea independently of the Gnathostomata.

In search for evidence the position of the intersegmental artery has been studied. This artery occupies in Petromyzontia the same position as in Gnathostomes relative to the dorsal and ventral nerve roots, passing up between the dorsal root of one segment and the ventral root of the segment next behind. But in adult Myxinoids, as shown by dissection and the reconstruction of transverse sections of *Myxine* kindly lent by Professor F. J. Cole, the artery runs up dorsally *between* the two roots which combine to form one mixed

nerve; that is to say, between the ventral root anteriorly and the dorsal root posteriorly.

That this artery is the true intersegmental vessel has been ascertained owing to the kindness of Professor G. C. Price, who lent sections of embryos of *Bdellostoma*. From quite early stages the artery occupies its definitive and unique position.

There seems to be no plausible way of explaining the difference between the relative positions of the nerve roots and intersegmental artery in the Myxinoids and Gnathostomes. Yet it may perhaps be assumed that this difference supports the view that the mixed spinal nerve has been independently acquired in the two groups. But the manner in which the difference has been brought about remains an unsolved morphological problem.

Professors Le Gros Clark, Wilson and Woollard discussed the significance of the intersegmental arteries and their ontogenetic development: they inquired whether the ultimate Myxinoid intersegmental vessel was preceded by a vascular network, or whether it manifested unity of individuality *ab initio*. Professor Hill considered that a slight caudal dislocation of the dorsal spinal nerve root might result in the actual Myxinoid condition found to exist.

Professor Goodrich, replying, confessed our general ignorance concerning the earliest stages of the developing vascular pattern: most probably a plexiform arrangement of vessels did precede the formation of the actual intersegmental artery, but even so, the essential problem requiring explanation was the final position of that artery to the spinal nerve roots. Caudal shifting of the dorsal root was an untenable hypothesis, since it would necessarily involve profound errors of segmental innervation.

Professor Le Gros Clark exhibited, and made remarks on, casts of fossil human skulls recently discovered in Java, and also showed the original fragments of the Swanscombe skull and the corresponding endocranial cast. The Ngandong skulls (*Homo* or *Javanthropus soloensis*) have been previously described by Oppenoorth. (W. F. F. Oppenoorth: "De Vonst van Paleolithische Menschelijke Schedels op Java", *De Mijningenieur*, No. 6, June 1932.) They strongly resemble in their general features the skull of *Homo rhodesiensis*. Their geological age is uncertain, opinions ranging from late Pleistocene to Mesolithic antiquity. The Modjokerto skull, which was discovered in Java in 1936, has been described by von Koenigswald. (G. H. H. von Koenigswald: "Erste Mitteilung über einen fossilen Hominiden aus dem Altpleistocän Ostjavas," *Proc. Kon. Akad. Wet. Amst.* vol. xxxix, No. 8, 1936.) It is the skull of a small child of an estimated age of 2–6 years. The cranial capacity is small, probably about 700 c.c., while the supraorbital ridges are developed to a remarkable degree. The skull was found in deposits associated with the Djetis fauna which is indicative of late Pliocene or early Pleistocene Age. It is interesting to note that in several places in Middle and East Java, the strata containing the Djetis fauna are found below those containing the Trinil fauna. In other words, there is reason to believe that the Modjokerto skull antedates *Pithecanthropus*.

The Swanscombe skull was found in 1935 and 1936 by Mr Marston in the stratified gravels of the 100 ft. terrace of the Thames. With it were associated a warm, interglacial fauna, and implements of middle Acheulian culture. A

report on this discovery by Mr Marston will shortly appear in the *Journal of the Royal Anthropological Institute.*

Professor DERRY commented upon the brow region of the Modjokerto child's skull, and stressed the need of caution in assessing age from suture-closure. Professor BRASH inquired whether this specimen exhibited any suggestion of microcephaly: Professor KIRK asked whether a mastoid process was present. Dr DE BEER considered the anatomical characters of this skull to substantiate Bolk's theory of retardation. In reply, it was stated that no mastoid was present and that microcephaly was not in question: Dr DE BEER's interpretation of the anatomical evidence was considered quite justifiable.

Dr L. H. DUDLEY BUXTON read a paper entitled "New interpretation of platymeria and platycnemia". The accepted causes for platymeria and platycnemia are posture and gait, though there is no exact agreement as to how different forms of posture can cause this flattening. An investigation of a series of long bones showed that if the mean values of the indices of flattening are compared there is a high correlation between the flattening of the femur and of the humerus. This correlation is too high to be the result of accident. Low indices of both bones are found among primitive peoples and high among civilized. It is suggested that if the bones of both extremities show a similar flattening neither posture nor gait can be the cause. Tentatively the suggestion is made that among primitive peoples the bones are more plastic in early life and therefore more easily influenced by muscular action. The cause of this plasticity is uncertain, but possibly some sort of deficiency, calcium or otherwise, might be responsible.

It should be noted that while the interclass correlation is so high the intraclass calculated on a series of Romano-British skeletons is small except between the flattening of the femur and tibia, so that there must be some different factor at work within the group and between groups.

The effect of side, age and sex was also discussed.

Professor DERRY remarked that Manouvrier's theory of platycnemia had long been abandoned, even by its author: the tibialis posticus muscle could not be held accountable for the condition, which he considered a problem of nutritional factors entirely. Dr GRAY recalled the recent demonstration that "boomerang tibia" in the Australian aboriginal was a pathological condition (being the result of framboesia). Was platycnemia of somewhat similar, if milder, pathological origin? Professor APPLETON insisted that postural factors were extremely significant, and much more so than the merely dietetic. Professor LE GROS CLARK stressed the concomitant and demonstrable flattening of the long arm bones in skeletons exhibiting flattening of femur and tibia, a feature which favoured the dietetic rather than the postural theory of causation. Dr MACCONAILL inquired whether any evidence could be adduced indicative of a causal dietetic vitamin deficiency.

Dr DUDLEY BUXTON in reply agreed that "boomerang" tibia was frankly a pathological condition: he considered that though flattening of the long bones of the arm negatives the postural causation of platymeria and platycnemia, yet nevertheless, posture must play an important role: platycnemic tibiae, for instance, were commonly associated with "squatting facets" on talus and tibia.

Dr A. E. BARCLAY showed a series of lantern slides to illustrate the "Effect

of posture on the position of the diaphragm". The slides demonstrated (1) the normal position of the diaphragm; (2) the extremes of its excursion; (3) the effect of laying the subject on the right and left sides (in the former case the diaphragm swung on a mesial axis, so that the right side was much higher in the chest than the left); (4) the effect of the patient hanging upside down. This showed that for a few moments the diaphragm retained the same level as in the vertical position, but that fatigue rapidly developed and the diaphragm level was then considerably raised. The "posture" of the diaphragm was attributable to tonic action.

An X-ray ciné-film of the movements of the diaphragm was also shown. The features to which Dr BARCLAY called attention were: (1) that the shape of the dome of the diaphragm altered very little, even in the extremes of respiration; (2) the costo-phrenic angle, the angle between the diaphragm and the chest wall, the lower limit of which is a potential space that is opened and closed with the expansion of the lung. Attention was drawn to the fact that this potential space was lower behind than elsewhere and to the clinical implications of this. Lantern slides were shown of a subject in whom the limits of the costo-phrenic angle had been marked out on the skin in the mid phase of respiration and also in the extremes.

Dr A. E. BARCLAY and Dr K. J. FRANKLIN presented a "Ciné-film showing the joint movements". This consisted of a series of still radiographs illustrating the normal and extremes of movement, and followed in each case by a cinematographic X-ray in which the actual movements were shown.

The film was compiled and presented in order to ask the question whether such a film would be of use for teaching purposes.

The way in which the film was received suggested that the answer was in the affirmative, and Dr BARCLAY and Dr FRANKLIN would, therefore, as soon as possible continue with the work and do what they could to improve it. When this was done copies would be available for anyone who wished to borrow or purchase.

Professor LOCKHART inquired concerning sexual differences of diaphragm levels as revealed by radiographical study: his experience had led him to believe that the female manifested a much greater diaphragmatic excursion than the male.

Professor APPLETON stressed the importance, in radiographic investigation of the chest, of the non-diaphragmatic factors: allowance must always be made for the very considerable costal movement, and for extension of the lumbar spine. Comparisons of the findings in the sexes were extremely difficult to evaluate.

Professor DAVIES recalled the great variation in level of the gall bladder and asked whether type of bodily habitus influenced recognizably the various levels of the diaphragm.

Professor MAINLAND commented upon the irregularity of outline of the right cupola of the diaphragm, so clearly shown in the film, and sought information concerning its meaning and production. Dr BARCLAY, in reply, said he had made no particular study of sex differences: women, however, made much better X-ray subjects than did male athletes, as the latter tended invariably to inhibit the diaphragm's excursion during screening. He agreed that in assessing diaphragm positions allowance must certainly be made for

movements of the thoracic cage and lumbar spine: moreover, the range of diaphragmatic level was most profoundly influenced by the tone and voluntary contraction of the abdominal wall muscles. The well-known variations in radiographic position of the gall bladder were probably directly associated, not so much with bodily habitus as with rotation of the liver in varying degrees: the point was not, however, capable of easy explanation. The irregular outline of the right diaphragmatic dome, though long known, was still inexplicable: theories of "muscle puckering" did not carry conviction.

Professor MAINLAND and Mr WHILLIS discussed various technical points in connexion with the making of films illustrating joint movements.

Dr M. A. MACCONAILL read a paper on "Stress and structure in synovial joints". The mechanisms for withstanding the effects of pressure stresses were summarized on the basis of previous work, and the tension-resisting mechanisms, tendons and ligaments, were described with particular reference to the microscopic structure of their collagen fibres, and the implications as to function to be drawn therefrom. The paper will be submitted for publication. Demonstrations were given of the microscopic and macroscopic form and arrangements of ligamentous tissues, as shown by polarized light.

The CHAIRMAN inquired whether the peculiar tissue conditions described were confined to joints; Professor DAVIES congratulated the author upon his demonstration of the anatomical basis underlying various well known physiological properties of joints; Dr INKSTER asked for a fuller explanation of "spin" as employed by the author in connexion with ligamentous structure; Dr STEWART compared this "spin" in ligaments to the spiral twist of woven cotton. Dr MACCONAILL replied.

Dr ROBERT WALMSLEY and Mr JOHN BRUCE read a paper on "Replacement of semilunar cartilages after excision". They stated that replacement of the semilunar cartilages of the knee-joint after operative excision had been observed in a series of adult dogs. The replacing mass has the general form of the excised cartilage and is derived from the articular capsule with which its peripheral margin is continuous. One year after the excision of the lateral semilunar cartilage, the new meniscus has a surface area greater than that of the normal cartilage, but it has not yet attained its thickness.

In a series of young rabbits the medial semilunar cartilages were excised and the rabbits were destroyed at 2-day intervals after the operation. Coronal sections were made of the entire knee-joints. Four days after the excision there is a well-marked cellular reaction in the synovial membrane at the medial side of the knee at the level of the plane of the joint. This proliferation of fibrous tissue cells extends throughout the medial part of the synovial membrane, and at the end of 10 days a small but definite meniscus has been formed. The new meniscus has, at first, upper and lower parts, but by about the twentieth day these have fused with each other. The new menisci, in their early stages at least, are formed of fibrous tissue and fibrous tissue alone.

Professor JOHNSTON asked whether a meniscus will regenerate in a fixed (e.g. splinted) knee-joint: Mr WHILLIS asked whether any microscopical examination had been made of the regenerated semilunar cartilage, and if so, what was the histological picture found. Dr STEWART emphasized the necessity of allowing experimental animals full freedom of activity after operation, as otherwise healing and regeneration were both much impaired.

Mr BRUCE, in reply, said no work had as yet been performed upon meniscus regeneration in a fixed joint: histologically the fibrous tissue matrix of the renewed cartilage showed very few cartilage cells at any time. He pointed out that this paper enshrined the first known experimental proof of the intra-articular sequelae following meniscectomy. The experimental findings were entirely in harmony with Dr MACCONAILL's conceptions of joint mechanics. Incidentally, in the dog, no evidence of any attempted regeneration of the excised patella was detectable at the end of 7 weeks.

Professor H. H. WOOLLARD and Dr J. A. HARPMAN read a paper on "The temporo-mandibular joint".

Dr P. BACSICH, on behalf of Mr E. P. STIBBE and himself, read a paper on "A simple Weigert technique on paraffin sections of C.N.S. material". The main objection against the straightforward Weigert technique is that: (1) the preliminary mordanting prohibits the use of any of the other main neuro-histological techniques on sections of the same material; (2) it makes the material hard and brittle, thus rendering the sectioning, especially the serial sectioning, very difficult; (3) the whole process is very long and laborious; (4) the double differentiation, which gives the clearest pictures, is uncertain, and in the case of serial sections does not give uniform results.

The authors' modification of the Weigert technique is as follows:

Part I. Preliminary treatment:

(1) Fix in 10 % formalin.

(2) Wash 24 hr. in running tap water.

(3) Embed *as far as* 2 % *celloidin*, then *throw* material in chloroform, change several times, and embed material through *benzol* in paraffin.

(4) Cut fifteen or twenty sections with rotary microtome.

(5) Mount sections on albuminized slides in the usual way.

(6) Dissolve paraffin in xylol (5 min.).

(7) Aether-alcohol (equal parts)—(2–3 min.).

(8) Cover whole slide with $\frac{1}{2}$ % celloidin (by pouring celloidin over slide and then dripping off the superfluous amount of solution).

(9) Put sections straightway in 70 % spirit (this hardens the celloidin and so the celloidin makes a protecting coat which will prevent the sections floating off during the staining).

(10) Distilled water (1–2 min.).

Part II. Staining:

(1) Mordant sections in 1 % of ferric-chloride (analytical reagent should be used) dissolved in 1 % of chromic acid solution for two hours in incubator at 36° C.

(2) Wash thoroughly (2–3 min.) in twice changed distilled water until sections are faintly yellow.

(3) Stain in freshly-made lithium haematoxylin for 2 hr. in incubator at 36° C. (10 c.c. of a 10 % solution of haematoxylin dissolved in 95 % spirit, 10 c.c. of concentrated watery solution of lithium carbonate, and 100 c.c. of distilled water).

(4) Wash thoroughly in twice-changed distilled water (2–3 min.).

(5) Tap water (add few drops of lithium carbonate concentrated watery solution) until the white and grey substances are clearly recognizable.

Usually there is an exceptionally clear differentiation between the grey and white matter, and no further differentiation is necessary. The nerve cells stand out on a pinkish purple background, and therefore no counter-staining is required, while the white substance is stained dark blue. Actually, during the staining process, *a self-differentiation* of the sections occurs. Frst of all, when sections are immersed in the staining bath, the grey matter becomes stained, and then, but only after a certain time (about half an hour), this staining gradually fades out, while simultaneously the white substance takes up the stain.

If, by any chance, the staining is unsatisfactory, the sections can be completely bleached in Pal's solutions, then thoroughly washed, and the whole process of staining repeated. It is possible to bleach sections two or three times in succession, and restain them satisfactorily.

The above modification of the Weigert technique was successfully applied on human and various animal materials. Its simplicity and reliability make it very useful for class purposes, but its main importance is in the staining of serial sections of normal and pathological research material.

Professor Le Gros Clark compared the merits of the various accepted methods of c.n.s. staining: he did not think the proposed new method held out any obvious advantage or superiority over older methods.

Professor Blair spoke in recommendation of the new Weigert technique: personal experience of its use had impressed him very favourably, three types of slide being obtainable from a single block.

Mr Richardson drew attention to the advantages of a new celloidin solution ("Necol"), the general utility of which was further enhanced by its cheapness.

Dr Bacsich, replying, claimed a considerable time saving as the outstanding advantage of the technique described.

Dr P. Bacsich read a paper on "Histology in the dissecting room".

There is no need to stress the value of the actual dissecting-room subject in illustrating the differences in colour of the mucous membranes of the oesophagus and the stomach, and the sharp, well-defined border-line between the two in the cardiac region, easily recognizable with the naked eye and related to their different histological structure.

The rosy colour of the fungiform papillae on the living tongue may be related to the relative thinness of the stratified epithelium covering their surface.

The colour difference to the naked eye between the mucosa on the surface of the vaginal portion of the uterus and the cervical canal may again be referred to the histological structure. This enables the student to understand the so-called pseudo-erosions of the cervix, where parts of the portion covered by the thinner columnar epithelium are liable to misinterpretation.

Glomeruli of the kidney are just on the border-line of the naked-eye vision, while in acute nephritis the enlarged glomeruli are easily recognizable with the unaided eye as "small teardrops". The relation of the cortex and the medulla to each other, also the appearance of the medullary rays and convoluted columns, may be easily observed with the naked eye, or a small hand-lens, and further explained with the aid of the microscope.

The appearance of the lienal trabeculae and their relation to the arteries and veins may be studied with the hand-lens and this knowledge confirmed by the microscope.

The affinity of the medulla of the suprarenals to chromic salts may be easily demonstrated on free-hand sections of this organ, while at the same time the relation of the cortex and medulla to each other is studied and supported by a histological demonstration.

The spongy character of the mucous membrane of the nasal conchae may be easily observed on the dissecting-room subjects, and readily confirmed by the actual histological specimen showing the densely arranged blood vessels.

The student's dissection of the brain, soft palate, urogenital diaphragm, parotid gland, and scalp, becomes more instructive with the support of the corresponding microscopical sections.

Pathologists dealing exclusively with human material have to wait at least 24 hr. after death before commencing with the histological examination. This circumstance means that their sections show well-marked post-mortem changes as well as pathological ones, and considerable time has to be spent in demonstrating what is post-mortem change and what is pathological. As they are unable to examine their material at an earlier time after death, they consider that the normal histology course should meet this difficulty by introducing suitably treated sections of human material.

To cope with the situation, special demonstration classes in the dissecting room are advised. The freezing microtome and staining solutions necessary for the most customary and simple stainings are taken into the dissecting room. Having been given the gross anatomical descriptions of various organs, students are gradually carried towards the finer structural details recognizable with the naked eye. Recourse is then had to a small hand-lens, and all the finer details to be observed in such a simple fashion are related to the structure of the organs. In this way most of the basic principles of the microscopic anatomy of the organs were discussed before using histological specimens. A small piece of tissue is removed from the actual dissecting-room specimen, and with the aid of the freezing microtome sections are made and stained on the spot. Under the microscope the fine details of the organs are demonstrated, and referred to details recognized by the previous methods.

Even the actual microscopical slide is studied first with a low-power lens, and then further examined under the microscope, when gradually higher and higher magnifications are introduced.

(Colour photographs of unselected dissecting room material were shown in proof of the argument.)

Professors DAVIES and MAINLAND summarized their personal experiences of the teaching of histology in the dissecting room, and raised the further problem as to whether histology were not more appropriately taught under the anatomist rather than, as at present in many centres, under the physiologist. The CHAIRMAN stated the new ordinances of the University of London relevant to histological teaching, which gave the anatomist all the control desired.

Drs P. BACSICH and C. F. V. SMOUT read a paper on the "Foetal vessels of the human placenta".

The authors made corrosion specimens of the arterial system in fifty placentae.

According to the pattern made by the arteries they were able to divide their specimens into (1) a disperse type in which the blood vessels divided dichotomously as they entered the placenta, and (2) a magistral type in which the two arteries extended as far as the margin of the placenta before giving off branches of any size.

They found that twenty-seven of the specimens showed a symmetrical distribution and twenty-three of the specimens showed an assymmetrical distribution, e.g. one-third would be supplied by one artery and two-thirds by the other. They emphasize that whatever the relation of the areas supplied by the two arteries these vessels were always of equal calibre in the cord.

They found a constant communication between the two arteries as they entered the placenta. Forty-three specimens showed a well-marked transverse communicating branch, while in seven specimens the two vessels were fused.

There was no sign of a peripheral anastomosis and therefore it is believed that all the vessels to the placenta are end-arteries, which would explain the frequency of white infarction found in normal placentae at term.

The authors believe that the transverse communicating branch establishes an equal distribution of blood and regulates the pressure in the placenta and, acting as a buffer system, counteracts the effects of uterine contractions.

Dr W. Brandt communicated a paper on "Prenatal development of hair tracts in primates". The author described the phylogenetic significance of certain features of the hair tracts in foetal lemurs and monkeys, and in the foetal gibbon, chimpanzee and gorilla. In the lemurine stock *Chiromys* exhibits the most truly primitive hair pattern found among primate mammals. In the Pithecoid stock, certain anthropoid features are already foreshadowed, for instance, the typically anthropoid upward direction towards the olecranon of the hair tract along the ulnar border of the forearm: this feature, strikingly developed in the foetal gorilla, is already plainly indicated in *Cebus* and even more so in *Macaca*. This last species exhibits a convergent olecranon hair-whorl, a peculiarity preserved in the foetal chimpanzee. The author also demonstrated the arrangement of hairs on the median and lateral portions of the upper lip, and the pithecoid and anthropoid stages of the pubic hair pattern. The results of his investigations indicate that the study of the hair tracts is of considerable value in determining the phylogenetic relationships of the various subdivisions of the great Primate order.

Professor J. P. Hill praised the clarity and utility of this communication, the first attempt to utilize the hair tracts as a basis for classification. Dr Gray congratulated the author, whose work seemed to support Bolk's theory that hair tracts follow the growth of the skin. Professor Le Gros Clark discussed the growth of the upper lip in the light of its hair distribution, upon which point Professor Wilson also commented. Dr Brandt, in reply, said he had dealt thus far with the purely prenatal development of hair; the effects of the Lamarckian principles which Dr Gray had mentioned could be determined in adult material only. From its hair arrangement *Chiromys* appeared to be a truly primitive form.

Professor H. H. Woollard read a paper on "Experimental observations in the mesencephalic root of the 5th nerve".

Professor J. T. WILSON called attention to previous attempts at the elucidation of the problem outlined in this paper, in particular the work of Williams. Professor J. P. HILL inquired whether the non-medullated nerve endings depicted were truly epilemmar in nature; he was assured that they seemed so by every available test applied.

Professor H. H. WOOLLARD and Dr MATTHEW YOUNG read a paper on "Time and rate ossification in the carpal region". Radiograms were taken of the carpal bones of children between the ages of 5 and 9 years in the hope that the growth of these bones might be used as an objective measure of the nutritional state. As a preliminary investigation the bones of the hands, wrists and lower ends of the forearms in about seventy boys and forty girls between the above ages were photographed on an X-ray film. The areas of the carpal bones were measured by means of a planimeter. On the same films it was possible to measure also the area of the whole wrist region in the form of a quadrilateral. It was found that, age for age, more bone had developed in the girls than in the boys. The total area of the wrist (i.e. bones and soft parts) was, however, greater in the boys than in the girls. In both boys and girls the area of the carpal bones was larger upon the left side than upon the right.

The coefficient of variation was calculated for height, weight and the area of the carpalia; it was found that the carpal bone area was three times more variable than were height and weight.

The increments in the carpal area were graphed at yearly intervals; the graphs show very considerable irregularities. Coefficients of correlations were calculated between the carpal bone area and (*a*) the height, (*b*) the weight: in both cases the coefficient was high. The coefficient of correlation was similarly calculated for (*a*) body type and (*b*) eruption of teeth: in each instance it was extremely low.

This small sample drawn from the elementary school population of a London suburb suggests that changes in the area of the carpalia during school age might be used as an objective measure of nutrition. Height and weight are generally considered to be good measures of nutrition, and with these the carpal area is highly correlated. Its high coefficient of variation suggests that this area is extremely sensitive to environmental influences. Since it lends itself to the radiographic method, it facilitates the collection of permanent records of very large numbers of children.

Dr MACCONAILL commented upon variations due to racial features as affecting ossification rates. Professor LE GROS CLARK referred to certain American investigations of a nature akin to Professor WOOLLARD'S: in these the carpus had been found altogether too variable for employment as a standard of assessment of nutrition, and the distal epiphyses of radius and ulna had been substituted. The pressing need was for some accepted and reliable standard to demonstrate nutritional defects in single individuals. Professor APPLETON observed that the carpal ossificatory centres, among others, appeared earlier in large-bodied children than in those of lesser physique.

Dr ALICE B. CARLETON read a paper on "Lemurs and crocodiles", which was essentially a criticism of a monograph by Professor G. L. Sera on "The morphological characters of *Palaeopropithecus* and the primitive aquatic adaptation of mammals". Professor Sera's conclusions are rendered doubtful

by his inaccurate attribution of various limb bones to *Palaeopropithecus*. Of these, the humerus, radius and astragalus have already been shown by Lamberton to belong to *Megaladapis*; the femur and tibia belong most probably to *Hadropithecus stenognathus*; the "clavicle" is, evidently, the fibula of *Megaladapis grandidieri*, and the "capitate" described by Sera is quite certainly the astragalo-navicular bone of a crocodile.

Dr J. Z. Young read a paper entitled "Preganglionic innervation of the adrenal gland". In elasmobranch fishes it is sometimes possible to trace nerve fibres all the way from their origin in sympathetic cells to their endings around chromophil cells. This condition is contrary to the scheme of preganglionic innervation of this tissue suggested by Elliott, and in order to test the question again in cats, the critical experiment of sectioning nerve roots and looking for degeneration in the adrenal medulla was performed. It was found that Elliott's hypothesis is correct and that degeneration of the finest fibres in the medulla occurs after section of the appropriate spinal roots. This also agrees with the observations of Hollinshead, published after the present experiments were completed.

By section of some only of the roots through which the fibres pass partial denervation of the medulla was obtained. No evidence was seen of the existence of a network of anastomosing fibres at the periphery: section of a few nerve roots produced well-localized areas of degeneration. Segmental relations are preserved in the innervation of the chromophil tissue, the front part of the medulla receiving fibres from the more anterior, the hind part from the more posterior spinal segments. The roots over which the fibres for the adrenal were found to emerge were D 6 to D 16 inclusive.

By section of appropriate parts of the splanchnic complex it was found that about half of the fibres for the adrenal medulla run in the greater splanchnic nerves, most of the rest in the lesser splanchnic and a few in more posterior branches from the sympathetic chain.

Professor Woollard inquired as to any possible evidence of the continuity of peripheral sympathetic fibres in the adrenal.

Professor J. T. Wilson commented upon the beautiful demonstration of the homology between chromaffin cells and sympathetic cells. Concerning nerve networks he recalled Bozle's work in coelenterate material: in these forms the continuity of the nerve network was merely apparent, no such genuine arrangement having been ever satisfactorily established.

Dr Bacsich referred to Botar's researches which stood in agreement with the author's present findings.

Dr S. Zuckerman read papers on "Differential growth of the prostate" and (with A. N. Gutkelch and C. C. Sandys) upon "Effect of sex hormones on growth of seminal vesicles". These papers will be submitted for publication in the *Journal*.

In the subsequent discussion Mr Bruce stressed the necessity for caution in applying the results of monkey experiments to the interpretation of clinical conditions in man. Clinicians now recognized various types of prostatic hypertrophy; enlargements of the so-called middle lobe (tumours of uterus masculinus region) formed only 30 % of the cases of enlarged prostate. Microscopical examination of certain excised hypertrophied human prostates clearly

revealed an epithelial hyperplasia, without any increase of the prostatic muscular element.

Dr ZUCKERMAN, replying, remarked that prostatic hypertrophy is of epithelial nature but of uterine morphology; true epithelial as well as muscular hypertrophy is obtainable experimentally: the prostate proper is dislodged backwards. Enlargements in the uterus masculinus region were adenomatous in nature: such adenomata did not usually reveal in microscopy any uterus masculinus, but the presence or absence thereof in sections was relatively unimportant.

Dr E. W. WALLS read a paper on an "Embryo, with placenta, of *Myrmecophaga jubata*". The specimen was collected by AGAR in 1908, and a previous account of the embryo has been given by MARSHALL (1920). A wax-plate reconstruction was made of the embryo, which was found to exhibit striking similarity to the human embryo at the 10 mm. stage. The placenta is discoidal in shape, the decidua showing well marked spongy and compact layers; in type it is villous-haemochorial. In the umbilical cord there persists the remnant of the allantois. The whole placenta belongs to group 4 of GROSSER's table, i.e. to the same category as the human organ: it is allantoic-deciduate and villous-haemochorial.

Professor HILL tendered congratulations to the author, as the placenta of this particular species had been hitherto undescribed. Its features were strikingly similar to those of the placentae of *Dasypus* and *Tatusia*, the connecting stalks in all these forms being much alike and curiously reminiscent of the human condition—a remarkable example of parallelism in evolution. The villous condition of the placenta in *Myrmecophaga*, in *Dasypus* and Edentates generally again recalled the features in the human organ.

Professor BLAIR thought the degree of vascularization of the villi present in this embryo to be much less than obtains in the human embryo of corresponding age.

Dr G. M. WYBURN read a paper on "The development of the supra-umbilical portion of the anterior abdominal wall". The supra-umbilical portion of the anterior abdominal wall is formed from the mesoderm of the anterior mesodermal field, which, following pericardial inversion, becomes the ventral part of a mesodermal mass, forming a partition between the abdominal and thoracic parietes. Into the dorsal half of this mesoderm projects the early hepatic bud which in its later growth extends ventrally and caudally and actually appears to push before it the abdominal parietes.

However, a liver normal in size and development does not necessarily predestinate a normal supra-umbilical abdominal wall as in cases of eventration with deficiency or total absence of this part of the parietes there may be a normally developed liver.

Dr A. J. E. CAVE read a paper on "The antiquity of vertebral nomenclature".

The Egyptians were the first people habitually to familiarize themselves with the internal structure of the human body, and for nearly thirty-six centuries were possessed, through their mummification rituals, of anatomical knowledge known to no other people of antiquity. The embalmers were not, however, anatomists in any scientific sense. They removed, handled and

preserved the viscera and body parts in manipulative procedures directed solely towards one end—the restoration and perpetual preservation of the body in its natural entirety.

Their knowledge of topographical anatomy must have been extensive, though no means exist of estimating it; the complete evisceration and preservation of the cadaver through a flank incision of but 90–110 mm. bespeaks an intimate acquaintance with the disposition of the internal organs. No anatomical papyrus has ever been found; Egyptian anatomical nomenclature is known from the lists of body-parts which occur as follows:

On the walls of the Saqqara pyramids (*Pyr. Texts*, 135; 148–9; 1303–1315), on Middle Kingdom coffins (*Coffin of Amamu*, XXIV, 11–18; P. Lacau, *Textes Religieuses*, XXVII), on the walls of Theban royal tombs (see E. Naville, *Litanie au Soleil*, pls. 14, 20, 21, 32), in the *Book of the Dead* (42; 172), *Book of Breathings*, and other funerary compositions, in the New Kingdom magical papyri (*Pap. Berlin*, 3027, 3, 6–5, 2; verso 4, 8–5, 5; *Pap. Turin*, 125, 5–11; *Pap. Leiden*, 348, verso, 5, 1–6, 2; *Pap. Chester-Beatty*, VII, verso 2, 5–5, 6; *Pap. Ch. Beatty*, VIII, 7, 1–9, 6; *Pap. Vatican*, 36, on the 30th dynasty *Metternichstele*, II, 15–32), in the *Tanis Sign Papyrus*, 7, 11–10, 6, on various ostraca, and elsewhere.

In these lists occur the vertebral terms discussed below, often followed by the most apposite and unmistakable determinatives—the oldest extant vertebral pictures known to history. The Egyptians were the first comparative anatomists, for they recognized the essential homology between animal and human organs and body parts in their habitual employment of hieroglyphs of animal origin for the several parts and organs of human anatomy. To them must be credited a priority in anatomical nomenclature, as well as that centuries old unprejudiced handling of the cadaver which ultimately made possible the rise of the Greek Alexandrian School.

(1) *Bones.* Egyptian records contain no word for (or picture of) the skeleton; the dead man, after mummification, was reanimated and therefore, ritually at least, alive again. W. R. Dawson (*J. Egypt. Archaeol.* 1924, vol. x (1), p. 1) has figured a unique vignette from the Book of the Dead (*Papyrus Louvre*) which apparently represents the shrunken corpse during the embalming process, but is not an actual skeleton. The word *Ḳs* [ ] is a general word for "bone", "bones", or the general osseous body framework. It occurs in Old, Middle and New Kingdoms, and full accounts of its derivation and meaning are given by A. H. Gardiner (*Egyptian Grammar* (Sign-list), 1927) and in more detail by F. Ll. Griffith (*Beni Hasan*, 1896, III, 24; *Hieroglyphs*, 1898, 52; *Mastaba of Ptah-hetep*, 1900, 1, 34). The object depicted in the determinative for *Ḳs* is a bone harpoon-head fixed in a wooden shaft. As a simple determinative it is confined to words describing bone or bone-like objects (e.g. *ỉib*, "ivory") and for expressing the meaning of "tubular" as in *mỉwt*, "shaft" (originally a hollow reed) and *twr*, "reed", and hence to words containing the syllable *twr*, e.g. *twri*, "to be pure". As a single sign *Ḳs*, often with the flesh determinative, means bone or bones of human beings though sometimes of animals such as hippo, crocodile, cat, snake, bird and fish. It occurs in funerary spells from the Pyramid Age to the New Kingdom.

Most significant is the association of the idea of hollowness with bones in general, as indicating an acquaintance with the medullary cavity of long bones.

(2) *Back* (*superficially*). The word *dpt* [], though used for "back", seems to be a non-specific general term of description. It is applied to regions other than the dorsal, and seems to indicate the muscular prominences of the body. The 20th Dynasty *Pap. Turin* has (125, 8), "the *dpt* of the back" (i.e. of the backbone), and the suggestion put forward here is that *dpt*, in this passage, refers to the subcutaneous eminences produced by the erector spinae muscles, bounding the vertebral furrow. Such an interpretation accords well with the sense of *dpt* in other connexions. It is not unlikely that *dpt*, originally referring to muscle masses, was later occasionally transferred by accident or carelessness to the back, in the external sense only. Its employment for "backbone" can be a corruption only.

(3) *Back, spine, dorsum of body.* The usual words for "back", "spine" are *iȝt* [ ] and *pśd* [ ] (older *pśdj*) [ ]. They have various determinatives, sometimes the general flesh-sign but more often the conventionalized back-bone with the spinal cord issuing from it. *iȝt* means "back", i.e. dorsum of body generally. It is interchangeable with *pśd*, the two terms being more or less synonymous. In the so-called *Ritual of Embalming* the word is used simply in the sense of dorsal aspect of the trunk (*Pap. Boulaq*, III, 2, 11). In *Pap. Ebers* (97, 10) occurs a magical injunction to bring milk to a nursing woman: the backbone of a silurus fish is to be cooked in fat and then rubbed upon the woman's backbone, on principles of sympathetic magic. Here, the back determinative might equally stand for *iȝt* or *pśd*. Both *iȝt* and *pśd* are interchangeable and are used indiscriminately for "back" (externally) and "spine". One or other occurs in all the lists of body parts, whilst both occur in the *Book of the Dead* (XLII). Occasionally they occur in parallelism, as in a magical spell in the *Toronto Ostracon* (1, 1–5) where the fiend is addressed thus: "Thou dost not take up thy position on his back (*pśd*) so that there is injury to his spine (*iȝt*)", and in *Pap. Leiden* (I, 348, verso, 5, 8) we have "his back (*pśd*) is the spine (*iȝt*) of Geb". Curiously these two words do not occur in the medical texts (save the *Edwin Smith Papyrus*, which deals with vertebral injuries). In the *Ebers Papyrus pśd* occurs but once, as a symptom of some abdominal disorder, thus: "Thou findest it (the disease) on his back (*pśd*) like a weight" (*Ebers*, 40, 6).

The word *pśd* (older *pśdj*) occurs commonly in lists of body-parts as the customary term for "back" in the sense of dorsum of the body. It is, however, interchangeable with *iȝt* as noted, but can, by itself, mean the vertebral column proper as distinct from its topographical position. For in *Pyr.* §§ 1544–8, wherein the dead King is represented as cut up like a sacrificial ox, each part being identified with a god, *pśd* is used for the backbone proper, having a most interesting determinative, the conventionalized vertebra, with typical elongated neural spine, of an ungulate animal (probably ox).

(4) *Vertebra.* The usual Egyptian word for "vertebra" is *ṯs* (*ṯs·t*) [ ], sometimes with the general anatomical determinative ( ) added. The word really means a "knot" or "tie", being commonly employed for the knots tied in magical cords and strings, and is connected with the word *ṯs*, "to tie". The backbone therefore is envisaged as a continuous band containing a number of knots, or as a series of bones strung together like beads on a string—a very apt and striking conception. The word *ṯs* occurs in the list of body parts in the *Pyramid Texts* (§ 1308 *c*), but not in later lists. It is sometimes combined with the word *bḳsw*

[] (*q.v.*) occurring thus in a Middle Kingdom list of body parts (see P. Lacau, *Textes Religieuses*, XXVII, 47). In the *Pyramid Texts*, *ṯs-bḳsw* occurs without determinatives (§ 229), and yet again (*Pyr.* § 409) with most elaborate and expressive determinatives, which are clearly conventionalized vertebrae, and therefore constitute the earliest pictorial representation of the vertebral column yet known. Once, in a 19th Dynasty list of body parts in the Tombs of the Kings, *bḳsw* occurs alone, following *pśḍ*, "back". In *Pap. Vatican*, 36, in a list of body parts, occurs the phrase "the vertebrae of the backbone", i.e. all the vertebrae except the cervical. The *Edwin Smith Papyrus* (17, 15–18, four times) has *ṯs-pśḍ*, "vertebra of the spine".

The cervical vertebrae are variously referred to: thus, "his seven of his neck" (*Pyr.* § 511); "the seven vertebrae of his head", i.e. supporting the head (*Pap. Leiden*, I, 343, verso 5, 5); "seven vertebrae from the spine" (*Pap. Turin*, 134, 3). The cervical spine was obviously well known, both as to function and numerical constitution, as far back as the Pyramid Age, *circa* 3000 B.C.—a significant reflection.

In many cases the sense of *ṯst* or *ṯs-bḳsw* is evidently that of the spinal column as a whole, and not of individual vertebrae—as for example in *Pyr.* 238, "rise up on thy spine (*ṯs*)."

The word *ṯs* also occurs in the surgical cases of the *Edwin Smith Papyrus* (10, 3–8; 10, 8–12; 11, 1–9; 11, 9–17; 17, 15–19), case reports very fully and faithfully detailing the signs and symptoms of various vertebral lesions.

Another word for "vertebra", whether human or animal, is *bḳsw* [], which is frequently given the vertebral determinative []. In the *Pyramid Texts* this word is most curiously applied to the invertebrate locust, it being said (*Pyr.* 1772 *b*) that "He (the King) has come forth from the *bḳsw* of the locust". In this passage *bḳsw* is given the determinative of a distinctive conventionalized vertebra not unlike that commonly employed for the heart (*ȝb*). This similarity of determinatives led to the confusion of *bḳsw* (vertebra) with *bśk*, an archaic or poetical expression for the heart, encountered only in the *Pyramid Texts* and in the *Book of the Dead*.

In the last-named *bḳsw* occurs, corruptly, thrice, i.e. in Spell 31 (*Pap. Nu*, 1, 4, without determinative), in Spell 133 (*Pap. Nu*, 1, 4 with determinative), and in Spell 149 *a*. Budge's translation of the *Papyrus Nu* shows a very early corruption of the word, inasmuch as the determinative given is the weighing balance.

The word *bḳsw* occurs in the *Edwin Smith Papyrus* (10, 12–21; Breasted's "case 31") in the phrase "Directions for a dislocation in a vertebra (*bḳsw*) of the neck", but in the other cases of vertebral injury the word *ṯs* is employed.

The *Ebers Papyrus* gives two references to the *bḳsw* of a goose, both in connexion with remedies for the hair. Thus *Ebers*, 65, 15, has "blood of the *bḳsw* of a goose" as an ingredient in a prescription, whilst *Ebers*, 65, 9–10 has "tortoise-shell; *bḳsw* of a goose; cooked in fat: anoint therewith many times".

(5) *Spinal canal and cord.* The cord is called *ȝmḫ* (*imekh*) [], and has for determinative the conventionalized backbone with the medulla spinalis issuing therefrom as, for example, in the Middle Kingdom tomb of Horhotpe, wherein occurs (1, 172) the phrase, "comes the spinal cord (*imekh*) issuing from the spine of Osiris". In contracted form the word is applied, by transference, to the spinal canal also: the sun-god Re‘ is said to

pass through the *imekh* of a great serpent, whilst a spell (VII) of the *Book of the Dead* is called "spell for passing through the spinal canal of Apophis, which is void". In this spell *ṯs·t* ("vertebrae") is actually written, but the essential notion of the hollow neural canal is faithfully preserved.

The foremost authority upon Assyrian medical terminology and history, Dr R. Campbell Thompson, states that the Assyrian anatomical terms are not as yet fully worked out. The models of ovine livers used in hepatoscopy and the general standard attained in other branches of medicine and science, render a considerable Assyrian anatomical vocabulary highly probable. Four or five words are said to be known for the different parts of the spine (probably from the superficial aspect), but the vertebral column itself is clearly named as an entity—*eṣen ṣirn*, the "bone of the back" or "backbone".

Dr H. L. H. H. GREEN described the macroscopic features of the "Brain of *Ornithorhynchus*", at two developmental stages, by means of reconstruction models. The foetuses measured 28 and 80 mm. respectively along the dorsal contour from the tip of the snout to the tip of the tail.

In the older specimen, striking features included the presence of a deep endorhinal fissure (there was no ectorhinal fissure at this stage), the relatively small size of the olfactory bulbs and tubercles, the very small optic nerves, and the enormous trigeminal ganglion. The dominance of the trigeminal apparatus was reflected in the brain stem by (*a*) the tuberculum quinti, (*b*) the extensive longitudinal swelling caused by the descending root and nucleus of the fifth nerve, and (*c*) the eminentia trigemini, a well-marked tubercle projecting posteriorly from the cranial part of the floor of the fourth ventricle. In the cerebellar region a shallow postero-lateral fissure limited a flocculo-nodular lobe. The diencephalic roof was widely exposed on the surface, despite the large size of the cerebral hemispheres. The pineal rudiment was very small.

In the younger specimen, attention was drawn to the following features: (1) the paraphyseal region, with a well-developed median choroid plexus in the telencephalon which had not yet spread back to encroach upon the diencephalic roof, (2) the absence of any delimitation of the roof of the diencephalon from that of the mesencephalon; no pineal diverticulum was recognizable, (3) the three separate sulci in the side wall of the third ventricle, viz. S. dorsalis, S. medius and S. ventralis. The dorsal thalamus appeared as a prominent swelling and the subthalmus was continuous anteriorly with a small eminentia thalami, (4) the internal thickening which extended across the roof in the region of the isthmus contained a large cerebellar (trigeminal) commissure; no external sulcus was yet developed to mark the rhombo-mesencephalic boundary in the median plane; indefinite bilateral swellings represented the developing corpora cerebelli.

A full account of these specimens will be submitted for publication in the *Journal of Anatomy*.

Professor WILSON detailed the history of the two brains described, referring particularly to the early pioneer work begun years ago in Sydney upon the monotreme brain by his then demonstrator—the late Sir GRAFTON ELLIOT SMITH. The second specimen was donated by Professor J. P. HILL.

Professor T. B. JOHNSTON put forward a "Plea for the use of the scapular plane as the reference plane for movements at the shoulder joint". The scapular plane was adopted by Thane in the 10th edition of *Quain's Anatomy*, but it

had not come into general use. Surgical practice usually follows anatomical teaching, but in this case the surgeons have at last broken away and the aeroplane splint—now almost universally employed for the treatment of shoulder injuries in preference to the Middledorp splint, which treated such injuries in the coronal plane—is used to maintain the humerus in the scapular plane. Reference was made (1) to the observation of Martin (*Brit. J. Surg.* vol. xx, 1932) that when the humerus is raised to the vertical in the coronal plane, the bone undergoes a process of lateral rotation as it ascends; and (2) to the observation of Codman (*The Shoulder*, Boston, Mass., 1934) that when the arm is raised to the vertical in the sagittal plane the humerus undergoes a process of medial rotation as it ascends.

From these two observations, the accuracy of which can easily be confirmed, the inference was drawn that somewhere between the coronal and the sagittal planes there is a plane in which the humerus can be elevated to the vertical without undergoing any rotation in the process. The scapular plane fulfils this condition. Martin (*loc. cit.*) has shown also that, however the arm be elevated to the vertical, in the final position the medial epicondyle points forwards and slightly laterally. Since, when the arm is raised to the vertical moving in the scapular plane throughout, the medial epicondyle points forwards and slightly laterally and since the fully elevated humerus must always be in the scapular plane, it can be inferred that, despite any rotation which may occur during elevation, at the end of the movement the humerus is not rotated to either side. But the observations of both Martin and Codman would lead one to anticipate some degree of rotation in the fully elevated humerus, and both have endeavoured to prove that such a condition actually occurs. Codman has even stated that "You can prove that the completely elevated arm is in either extreme external rotation or in extreme internal rotation".

The actual observations quoted are all accurate and, though apparently contradictory, must be capable of being harmonized. The explanation lies in the fact that movement of the arm from the sagittal to the scapular plane passively imposes on the humerus approximately 60° of lateral rotation, and movement of the arm from the coronal to the scapular plane similarly imposes on the humerus approximately 30° of medial rotation. This can be demonstrated most convincingly by placing a disarticulated humerus in the position of right-angled flexion, using Thane's flexion plane (i.e. a plane at right angles to the scapular plane) and then swinging it into the scapular plane. If the bone is then adducted, it will be found to have undergone 90° of lateral rotation, although it has not been actually rotated around its own long axis. The rotation is imposed on the bone as it is swung from the flexion plane to the scapular plane, for in that movement the bone moves round a vertical axis passing through its head. If this lateral rotation were not counteracted by a process of active medial rotation, the supra-spinatus and the acromial fibres of the deltoid would be thrown out of alignment and their efficiency would be seriously diminished.

In a similar way, it can be shown that the active lateral rotation which accompanies elevation of the arm from the coronal plane is designed to counteract the medial rotation which the movement from the coronal to the sagittal plane imposes on the humerus; and so enables the supraspinatus and the acromial fibres of the deltoid to maintain their proper alignment throughout the movement.

Mr W. F. B. GRIFFITHS read a paper on "Early development of reptilian embryos".

Dr G. WEDDELL read a paper on "The anatomy of taste". This work was done in collaboration with Dr H. G. SCHWARTZ at the Neuro-surgical Clinic, Barnes Hospital at St Louis, Mo., U.S.A., under the direction of Dr E. SACHS. The scope of the inquiry was confined to the anterior two-thirds of the tongue in the human subject.

The generally accepted pathway of fibres transmitting the sensation of taste from the anterior two-thirds of the tongue, is that from the taste-bud fibres pass in the chorda tympani as far as the geniculate ganglion in which they have their cells of origin. Thence fibres pass to the brain stem via the nervus intermedius of Wrisberg.

However, several observers (MORRISTON DAVIES, CUSHING, CARMICHAEL and WOOLLARD *et al.*) have reported that anaesthesia over the trigeminal area following any of the ordinary procedures for the radical cure of trigeminal neuralgia may be accompanied by a loss of taste over the corresponding anterior two-thirds of the tongue.

The following observations may have some bearing on the above apparently contradictory facts.

Taste was carefully tested in a series of cases of major trigeminal neuralgia both before and after the operation of central root section by the lateral approach. (The motor root and some ophthalmic fibres were preserved.) These cases, along with others, can be divided into the following groups.

Group I. In these the operation was comparatively easy, the dura stripped up readily and there was no facial palsy following operation. No loss of taste occurred.

Group II. In these the operation was usually difficult, the dura being resistant and friable. There was no facial palsy but a temporary loss of taste in a few cases which usually recovered in a couple of weeks.

Group III. In these facial palsy followed the operation, which sometimes resembled those of Group II in its difficulty. Always there was loss of taste while the facial palsy lasted. The above findings are in accord with those found by other observers.

Group IV. A case in which the greater superficial petrosal nerve was sectioned at operation and a portion removed. It contained numerous large myelinated fibres. Subsequently the patient was found to have lost the sensation of taste over the anterior two-thirds of the tongue on the side of the lesion. There was no facial palsy. This subject was examined over a period of two months and the loss of taste persisted unchanged during this time.

Group V. Taste was then tested in a series of cases following radical mastoidectomy in which both ossicles had been recovered intact. In two out of six cases taste was wholly unaffected. In one case there was a partial loss of taste.

Finally, a series of temporal bones was examined, and the foramina for the chorda tympani were measured as well as the foramen of exit of the greater superficial petrosal nerve. Generally speaking the sizes of the foramina for the two nerves were inversely related. It is of great interest that in one bone the foramen of exit for the greater superficial petrosal nerve was a large one but no foramina could be found for the chorda tympani.

The hypothesis that in a certain number of cases the sensation of taste from

the anterior two-thirds of the tongue may pass to the geniculate ganglion via the greater superficial petrosal nerve is being examined by anatomical and experimental methods.

Professor WOOLLARD said loss of taste over the anterior two-thirds of the tongue occurred in about half the cases of removal of the Gasserian ganglion. Dr DIXON BOYD inquired as to the means employed by the speaker in testing loss of taste: salt, quinine-sulphate and sugar were the substances used.

Dr CARLETON was informed that no glossal ataxia supervened upon trigeminal ganglionectomy. Professor LE GROS CLARK inquired concerning the supposed pathway of taste fibres from the forepart of the tongue, after destruction of the chorda tympani: this point was the crux of the whole problem.

DEMONSTRATIONS

At this Meeting of the Society the following demonstrations were given:

(1) S. ZUCKERMAN: "Tissue proliferation displayed by the colchicine technique". A number of sections were demonstrated of tissues from monkeys that had been injected with sex hormones for varying periods prior to autopsy, immediately before which (up to 9 hr.) they had been given colchicine. The effect of the latter is to "fix" dividing cells, so that many more mitotic figures are seen than is usual in ordinary preparations.

(2) G. R. DE BEER: "Models illustrating presumptive morphogenetic zones in eggs of different vertebrates."

(3) B. D. PULLINGER and H. FLOREY: "Proliferation of lymphatic vessels."

(4) MATTHEW YOUNG: "Casts and photographs of the Swanscombe skull and of other fossil skulls."

(5) J. S. BAXTER and J. D. BOYD: "Human embryo of ten somites." This embryo was obtained from a case of unruptured tubal pregnancy. The history, briefly, is:

Menstruation	1–6. vi. 36;
Marriage	7. vi. 36;
Bleeding p.v. commenced	28. vi. 36;
Laparotomy	12. vii. 36.

Hence, if ovulation occurred on the 14th day of the cycle, the age of the embryo is about 27–28 days.

The chorionic vesicle measured 14 × 13 × 12 mm. Length of the embryo in fixative was 2 mm.; after dehydration, 1·7 mm. The yolk sac is torn at its attachment but apart from this the histological state of the embryo is good when compared with other somite human embryos. The sections themselves and models of the external form and of the heart of the embryo were demonstrated.

(6) H. M. C. CARLETON: (*a*) "Dioxane, a substitute for dehydration and clearing." Dioxane is useful as a substitute for both alcohol and clearing reagents preparatory to paraffin embedding. It has remarkable property of mixing both with water and with paraffin wax. Hence tissues are placed direct in dioxane after fixation. If fixed in fluids containing $HgCl_2$ a few flakes of iodine are added to the dioxane to remove mercury deposit. Thin (2–3 mm.

thick) pieces of tissue lose their water in a few hours; bulky objects require treatment overnight.

(*b*) "Some fallacies in connexion with the fixation of glycogen," showing that the best fixative would seem to be

Dioxane saturated with picric acid	85 c.c.
40 % formaldehyde	10 c.c.
Glacial acetic acid	5 c.c.

(7) M. A. MacConaill: "Structure of ligaments displayed by polarised light after clearing in syrup." A series of specimens was shown which had been cleared and mounted for examination without previous dehydration. The clearing fluid used is made by taking equal parts of best pale golden syrup and glucose syrup. Glucose syrup is also known as liquid glucose; it should be highly concentrated. The moieties of the syrups are placed together in a wide jar, not too deep; covered, and heated (37–50° C.) until completely liquid, and then well mixed by stirring and several inversions of the jar. The mixture is then put back for further heating at the same temperature, to drive off the larger air bubbles. An hour or so is sufficient for this, and the smaller bubbles will disappear after a day or two, on standing in the cold. The clearing syrup so made is lemon yellow in bulk, but is not sensibly coloured in thin layers. The organ or piece of tissue to be cleared is covered with the fluid, warm or cold, either in the fresh state or after fixation. The fluid is a preservative against bacterial or fungal destruction of tissue: a Petri dish containing both fluid and tissue was exposed to the air of the laboratory for 2 months, and then examined bacteriologically: neither moulds not fungi could be grown from it.

This clearing fluid is intended for the rapid macroscopic or low-power binocular microscopic examination of tissues by the research worker, and for the preparation of teaching specimens. Clearing of razor sections takes place in about 1 min.: thicker pieces take from half an hour to several hours, overnight for a 4-months foetus. The vessels or cavities of organs can be injected with coloured masses in the usual way, and thick sections from such material can be examined microscopically within half an hour from the beginning of the work. These specimens are particularly suitable for examination by reflected light, especially from a Pointolite or the like. Whole foetuses, or such parts as are hollow, as the skull, should be sectioned longitudinally, as in the Spalteholtz technique, before being cleared. This appears to be the only precaution required: this fluid, having a high osmotic pressure tends to cause distortion of hollow structures if penetration be from their external walls only.

The use of sugar solutions as mounting media is well known and has long been practised. The present medium has been devised upon first principles. Golden syrup (molasses) has quite marked clearing powers. Adding glucose syrup confers three properties upon the mixture. It makes a clearer, more transparent fluid; it makes a less watery fluid; and it prevents crystallization of the sugars, by reason of the high viscosity of the glucose component. Experiment shows that the mixture is in every way better than glucose alone, for glucose syrup is too viscous for easy working and too supersaturated for easy penetration of the tissues. The addition of the glucose syrup has yet another advantage. The fluid soon becomes so viscous at the edges of a cover-glass that it is an easy matter to seal off the edges of a permanent or semipermanent preparation, microscopic or macroscopic.

Tissues immersed in this syrup quickly become very hard. They can be taken out, drained of the surplus syrup, and then preserved in a candied state. When required again they are simply immersed once more in the clearing syrup. If desired these syrup preparations can be softened in water, cut up then as wanted, and recleared. Experiments in progress at the Glass Technology Department of Sheffield University show that such syrups as have been used above can be transformed into a rigid glassy state by a suitable degree of supercooling below room temperature. At room temperatures the state of the syrups may be considered as analogous to a "molten glassy" condition which retains some of the transparency and viscosity of the "rigid" glassy state. This fact may well help to explain the good results to be had by using these syrups conjointly as a clearing fluid.

(8) R. O'Connor: "Transplantation of pronephros in larval amphibia."

Technique. Photographs and microscopic slides were used to record some of the results of transplanting the presumptive pronephros in *Amblystoma tigrinum* and *Triton taeniatus*, transplants being made between embryos of the same species and from one species to the other. The colour difference between the two species was used to make certain the transplant had remained in position, and when embryos of the same species were used staining of either host or donor with nile blue sulphate was used for the same purpose. However the colour difference, whether natural or artificial, only persisted a few days and after that the only external indication of the transplant was some irregularity of the external form of the host.

Results of injury to the host pronephros. Some results were demonstrated of the effect of injuring the pronephros at an early stage of development. Although a considerable degree of damage was inflicted development continued but resulted in abnormal tubules. Either they had a greatly increased lumen with flattened cells or had undergone degeneration and in the latter case there was evidence of a definite amount of development before degeneration took place. In both cases study of serial sections showed disorganization of the segmental duct and an interruption of its continuity from pronephros to cloaca. This abnormal development gave rise to difficulties in interpreting the results of transplantations made into the pronephric region of the host, for when abnormal tubules arose it was not possible to tell whether they had developed from injured host pronephric tissue, or from the transplant.

Transplantations in heterotopic positions. Transplantations were therefore made into regions away from the host pronephros and an example was shown of a transplanted pronephric tubule growing in a region dorsal to the gills. The transplant was taken from *Amblystoma tigrinum* in the early tail bud stage and transplanted into *Triton taeniatus* at about the same stage of development. Sections made seven weeks later showed a pronephric tubule from the transplant lying immediately lateral to the spinal cord and partially replacing a spinal ganglion, thus demonstrating that pronephrenic tubules will develop in an abnormal position and remain recognizable for a period of seven weeks.

Transplants taken from the gastrula stage. Transplants were taken from both species in stages ranging from the beginning of gastrulation to those where movement had begun. Those from the gastrula stage were sufficiently large to include more tissue than the presumptive pronephric area. In these development of pronephric tubules could not be shown, although differentiation of

muscle, cartilage, spinal cord and notochord occurred. These four tissues were shown in *Amblystoma tigrinum* where they had developed from a transplant taken from the gastrula stage of *Triton taeniatus*. The significance of these findings will be the subject of further inquiry.

Incidental transplanting of the fore limb bud. The developing pronephros is in close relationship to the developing fore limb bud and hence it is difficult to avoid transplanting some of the limb bud with the pronephros. This fact gave rise to an interesting incidental finding in an experiment where the pronephric area and part of the fore limb bud was transplanted from *Amblystoma tigrinum* into the region above the gill rudiment of *Triton taeniatus*, both being in the tail bud stage. An abnormal limb developed and on section 8 weeks after the transplantation was made it was seen to have two humeri fusing distally. One could be traced to a scapula formed from the transplant and the other to the scapula of the host.

(9) P. Bacsich and C. F. V. Smout: "Museum specimens by celluloid corrosion technique." The vascular system of various organs was injected with a coloured or uncoloured solution of celluloid in acetone. The tissues were removed by maceration and in this way the vascular tree and channel system of the organs were demonstrated. The authors believe that these specimens would be a great help in teaching.

(10) Professor F. Davies demonstrated a method of exhibiting histological sections (Weigert-Pal) of the brain stem and spinal cord. Copies are made of drawings of sections (from such an atlas as that of Villiger) by means of Kodaline slow paper (no camera being necessary). These are mounted on cardboard and covered with glass. Attached to the lower edge of these are wooden boxes, whose front surface is formed by the slide with section mounted, and whose upper surface is formed by a blank slide. The slides are attached to the edges of the wooden box by passe-partout. Lying diagonally in the box is a sheet of white celluloid, so that when the box is illuminated from above the celluloid reflects the light through the section. By mounting a representative series of such sections on the museum wall, illuminated from above by a strip-light, they form a convenient series (with explanatory diagrams attached) for study of the structure of the cord and brain stem with a hand lens. The method is also commended by its cheapness.